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REDIFFERENTIATION OF NaCl TOLERANT SUGARCANE PLANTS FROM CALLUS DERIVED RESISTANT LINES

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SALT-resistant cell lines have already been selected in *Nicotiana sylvistris*[1], *N. tabaccum*[2], *Medicago sativa*[3], *Citrus sinensis*[4] and in some grain legumes[5]. However, in most cases regeneration of plants from resistant cell lines is difficult to achieve[6]. The selection of a sugarcane cell line tolerant to NaCl was earlier accomplished, but many problems in recovering an entire plant have been discussed[7]. The present communication reports a successful attempt of regenerated salt tolerant sugarcane plants from varieties Co740 and Co62175, those were mainly derived *in vitro* from the callus cells grown in high salt conditions. Salinity is an increasing problem in the sugarcane fields of India and these two cultivars are salt-sensitive[8]. Hence the selection and regeneration of a salt-resistant plants are of great importance.

The callus was raised from stem tip and young leaves on modified Murashige and Skoog[9] (MS) medium as suggested by Heinz and Mea[10]. The actively growing callus was subcultured at 15–30 day intervals.

For selection of salinity tolerant line the salt (NaCl) was added into the basal MS medium during subculturing of callus on agar medium. Salt concentrations at various levels starting from 0.1, 0.2, 0.5, 1.0, 1.5 and 2.0% were used.

The relative growth response of the callus cultures of sugarcane var. Co62175 and Co740 on MS medium with increasing concentration of NaCl is shown in figures 1–3. It can be seen that both varieties showed stimulation of the growth up to 0.2% of NaCl in basal MS medium, while the growth was inhibited beyond 0.5% NaCl and at about 1% the growth was completely inhibited. At this concentration the callus shows browning, compactness and subsequent death of the tissue. Nevertheless, small sectors of good growing tolerant cells were observed in some culture tubes even at a higher NaCl level (1.5%). These actively growing cell aggregates were then isolated and subcultured

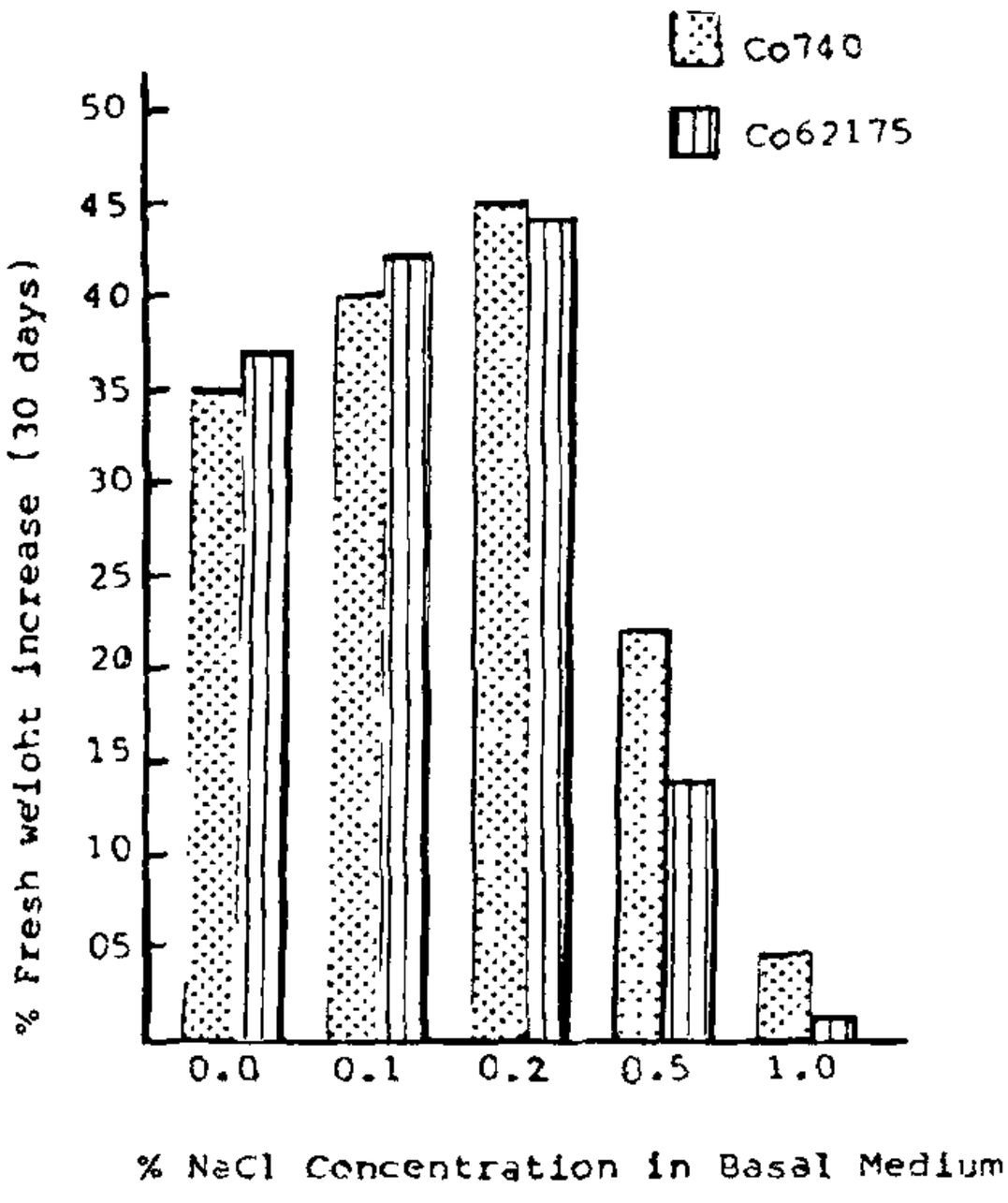
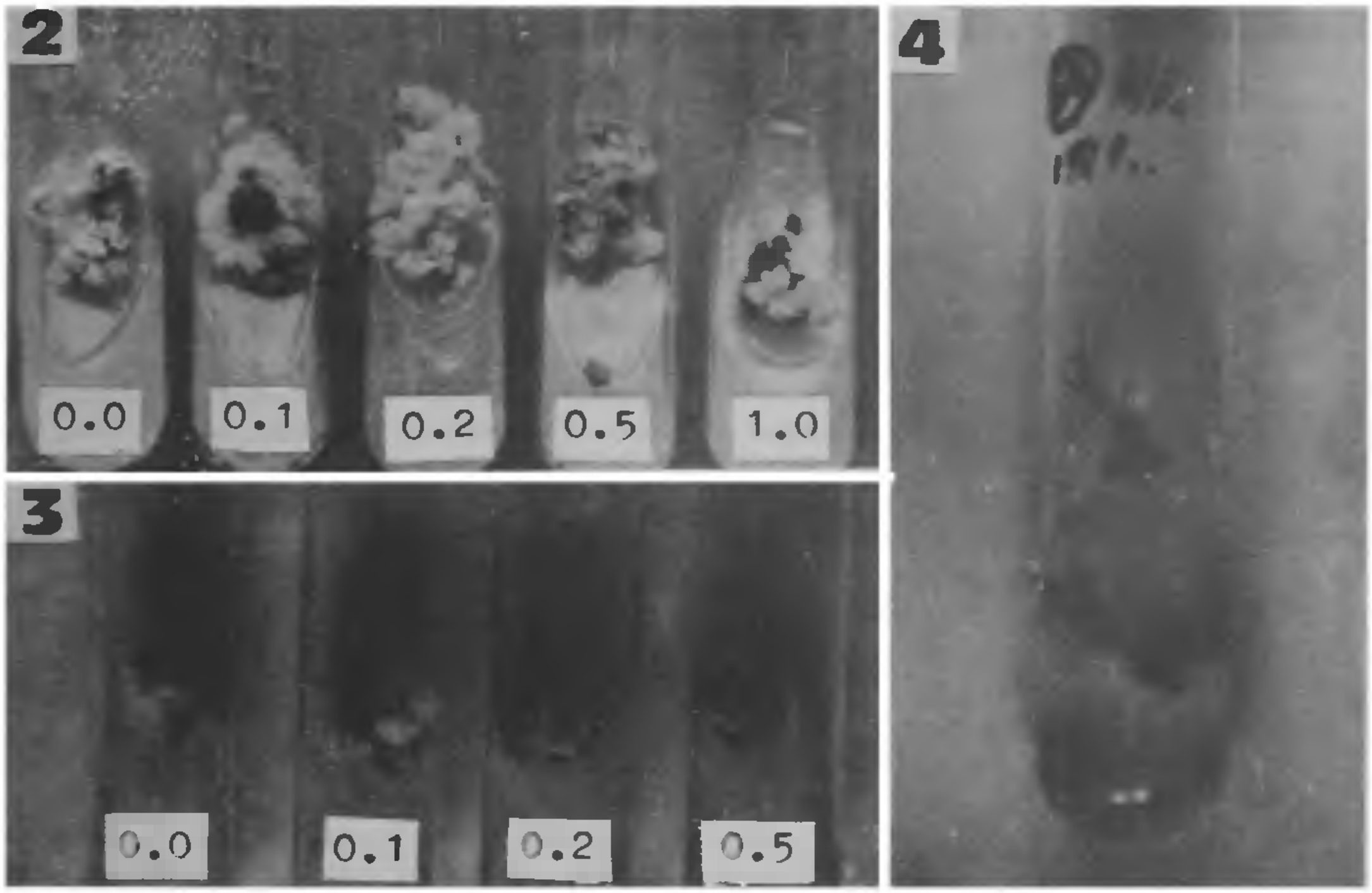


Figure 1. Growth of sugarcane callus in different salt concentrations.



Figures 2–4. Growth of callus of sugarcane; **2.** Var. Co740 in different salt concentrations; **3.** Var. Co62175 in different salt concentrations; **4.** A regenerated plantlet of var. Co62175 in 1% salt medium.

on medium with 1.0, 1.5 and 2.0% NaCl levels. Likewise continuous subculturing of these selected cell lines at an interval of 30 days resulted in obtaining callus-derived salt-tolerant cell line in these two varieties of sugarcane (Co740 and Co62175).

The selected callus was further transferred on to the modified MS medium with and without cytokinine for shoot initiation at 26 ± 2°C with 12 h illumination at about 1000 lux. The medium without 2, 4–D was found to be useful in inducing shoot primordia (42%). Further, the addition of 0.5 mg/l benzyl aminopurine (BAP) induced a greater number of shoots (65%) even at higher concentration of NaCl. The present results show that the regeneration capacity of selected cell line of var Co62175 is higher as compared to Co740. The rooting of shoots was induced in MS medium supplimented with 0.1 mg/l indole butyric acid (IBA) and 0.05 mg/l naphthalene acetic acid (NAA). After induction of shoots and roots and further subculturing in high salt medium, a complete regenerated plantlet growing in 1% NaCl was obtained in var Co62175 (figure 4). The plantlet was subcultured three times and a continuous but slow growth is noticed.

Similar redifferentiated plants have earlier been obtained from salinity-tolerant line in *Tobacco*[2], *Alfalfa*[11] and *Datura innoxia*[12]. However in these reports also the selected and regenerated plantlets have shown weak and slow growth. Liu and Yeh[7] reported that in the selection procedure for salinity tolerant line the callus cells lose their regenerating ability. Thus, it is an interesting attempt to obtain a redifferentiated plant which is growing on 1% NaCl medium. Yasuda *et al*[13] have also claimed the regeneration of a plant from a callus initiated sugarcane plantlet grown on NaCl containing medium. The present report shows similar success in Indian sugarcane varieties.

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EFFECT OF STORAGE TEMPERATURE ON VITAMIN C CONTENT OF MUSHROOMS (*AGARICUS BISPORUS*)

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MUSHROOMS are generally consumed for their flavour and texture but their nutritive value has been appreciated only recently. They constitute a good source[1–3] of vitamin C. Very few studies are available on vitamin C content of mushrooms and there is a wide variation in the reported values (2.6 to 788 mg/100 g). Besides, mushrooms have short shelf-life at ordinary temperature but can be stored for several days in a refrigerator. Changes in vitamin C levels in mushrooms during post-harvest storage have been studied in the present investigation (vitamin C includes both ascorbic acid and its oxidation product, dehydroascorbic acid (DHA) as both are antiscorbutic).

Button mushrooms (3.5 ± 0.2 cm cap diameter), packed (200 g lots) in 100 guage perforated polyethylene packets were stored in B.O.D. incubators at 5°C (85–90% RH), 10°C (70–75% RH) and 15°C (55–60% RH). Four replications were maintained. Samples were drawn every day till the 4th day. For analysis, one longitudinal quarter from each mushroom was pooled; 20 g of the pooled sample were extracted twice with a mixture of 5% metaphosphoric acid + 10% acetic acid and filtered on a sintered Buchner funnel (porosity G. 2). The filtrate was analysed for the total vitamin C content, ascorbic acid and dehydroascorbic acid by dinitrophenyl hydrazine method using acid washed Norit as oxidant[4].

Immediately after harvesting, the mushrooms contained about 83 μg vitamin C (80 μg ascorbic acid + 3 μg DHA) per gram fresh weight. Similar values have been reported by other workers[2,5]. Almost all the vitamin C in fresh mushrooms was in the reduced form as in other vegetables and fruits[4,6]. After 4 days of storage the mushrooms lost about 12–25% of vitamin C, the loss being greater at 15°C, but major changes were noticed in the proportion of reduced: oxidized forms of vitamin C. Decrease in ascorbic acid was accompanied with increase in DHA, and higher storage temperature had profound effect. The above changes, not significant up to 24 h, increased significantly with time (figure 1).

Ascorbic acid is oxidized to dehydroascorbic acid by many oxidases especially the ascorbic acid oxidase which catalyses direct oxidation using molecular oxygen. Other oxidases cause indirect oxidation i.e. through their oxidation products. DHA is reduced to ascorbic acid by DHA reductase using glutathione. The oxidation of ascorbic acid to DHA, therefore does not represent the loss of vitamin C as both forms are antiscorbutic. The loss of the vitamin occurs due to further conversion of DHA to diketogulonic acid (DKA), which is irreversible. The reaction is highly pH-dependent, slow

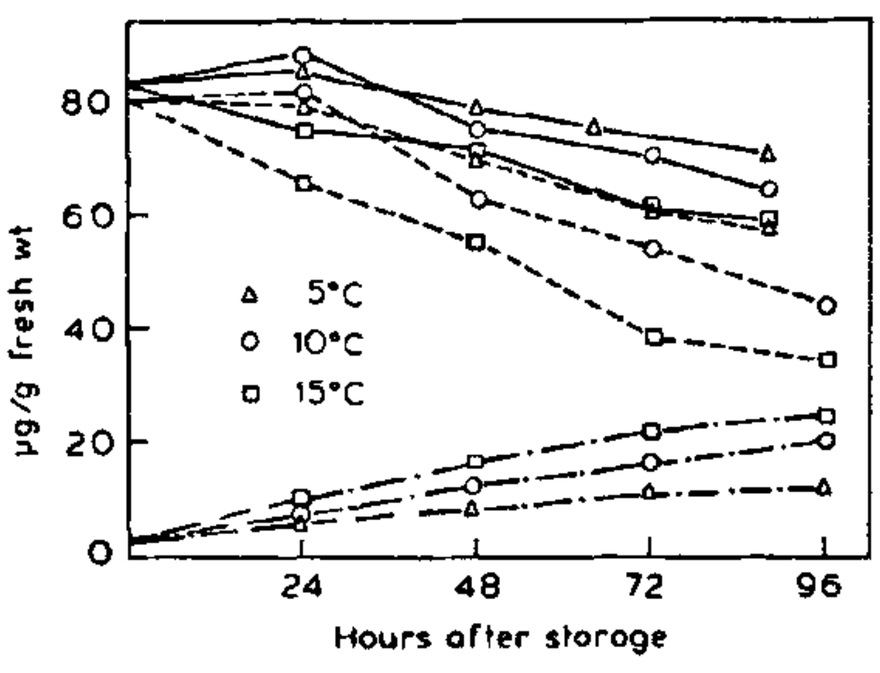

Figure 1. Effect of storage temperature on the contents (μg per g fresh weight) of total vitamin C (———), ascorbic acid (— — —) and dehydroascorbic acid (—·—·—) in mushrooms (*Agaricus bisporus*).