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Sodium Chloride Resistant Cell Line from Haploid *Datura innoxia* Mill.

A Resistance Trait Carried from Cell to Plantlet and vice versa *in Vitro*

Brief Report

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Summary

A cell line resistant to sodium chloride was selected from callus cultures of haploid *Datura innoxia* by cloning under selective pressure. Cells of the resistant cell line retained their resistance even after subculture in absence of NaCl. Plantlets could be regenerated from resistant cells in the presence as well as absence of NaCl. In contrast, regeneration of plantlets was not possible from normal cells in the presence of NaCl, although regeneration readily occurred in the absence of NaCl.

To examine the stability of the resistance in the long-term, callus cultures were initiated in presence of NaCl from stem explants of the differentiated plantlets. All explants of plantlets derived from resistant cells showed callus formation. This callus, derived from resistant explants, retained the trait of resistance upon subculture.

Keywords: Callus culture; *Datura innoxia;* Plantlet regeneration; Sodium chloride resistance.

1. Introduction

Increasing attention is being focused on the application of cell culture techniques to select desirable mutants and regenerate plants possessing useful traits (see MALIGA 1978). Selection of resistant cell lines for adaptation against various environmental stresses like salt tolerance is advantageous not only from the standpoint of economic and human welfare but also for facilitation

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of experimentation to understand physiology of salt tolerance (ZENK 1974). Cell lines resistant to deleterious concentrations of sodium chloride have already been selected in *Nicotiana sylvestris* (ZENK 1974, DIX and STREET 1975), *N. tabacum* (NABORS, DANIELS, NADOLNY, and BROWN 1975), *Capsicum annuum* (DIX and STREET 1975), and *Citrus sinensis* (KOCHBA, SPIEGEL-ROY, SAAD, and NEUMANN 1978). However, in most reports, the experiments have not been pursued in detail and regeneration of plants from resistant cell line has been reported only recently in *N. tabacum* (NABORS, GIBBS, BERNSTEIN, and MEIS 1980).

We report here the selection of a NaCl-resistant cell line in callus cultures raised from a haploid plantlet of *Datura innoxia*. The calli raised from stem explants of plantlets, regenerated from resistant cell line, retained their trait of resistance to NaCl.

2. Material and Methods

Callus cultures, raised from a haploid plant of *Datura innoxia* Mill., were used for the experiments described in this report. MS medium (MURASHIGE and SKOOG 1962) containing 2×10^{-5} M $FeSO_4$ and Na_2EDTA as the iron source and 2% sucrose was used in all experiments. In experiments concerned with callusing, 2,4-dichlorophenoxyacetic acid (2,4-D) at 9×10^{-6} M was used alone or with 10^{-7} M benzylaminopurine (BAP). Regeneration of plantlets was achieved on differentiation medium comprising 10^{-6} M BAP and 10^{-5} M indoleacetic acid (IAA). When necessary, sodium chloride was added to the medium before autoclaving. The pH was adjusted to 5.7 with 0.1 N NaOH. Callus cultures were kept in diffuse light (1,000 lux) and regeneration of plantlets was achieved in cultures kept in stronger light (4,000 lux) provided by Cool Daylight Fluorescent tubes (Philips, TL 40 W/54) at 26 ± 1 °C during the 16-hour photoperiod and 23 ± 1 °C during the dark period.

A simplified scheme of major procedural steps in selecting salt-resistant line has been given in Fig. 1. For selection of resistant cell line, 50 mg pieces of callus were inoculated on agar-gelled medium containing 1% NaCl. After one month tissue proliferation was observed. Only four cultures showed growth from defined regions of callus which could be distinguished from neighbouring necrotic tissue. These growing sectors were subcultured monthly till uniform growth was observed. After three subcultures, one cell line showing the maximal growth was selected and regularly subcultured at intervals of about one month.

For studies of growth, callus pieces each containing 10^5 cells were inoculated on agar-gelled medium at varying concentrations viz. 0, 0.5, 1.0, 1.5, and 2.0% of NaCl. Fresh weight increase was recorded after one month of growth and data were converted into increase in number of cells. The number of cells per unit fresh weight could be determined in approximate terms by macerating a known amount of callus in 5% chromic acid and then counting cells with the help of a haemocytometer. Representative calli from each treatment were used to determine the number of cells per unit fresh weight. Simultaneously, controls were kept to study the effect of NaCl on normal callus.

For plantlet regeneration, small pieces of callus were inoculated on the medium with and without 1% NaCl. Callus was initiated from stem explants of the differentiated plantlets on callusing medium. BAP at 10^{-7} M concentration was also added to this medium during induction of callus as in our experience without simultaneous presence of BAP callusing was very limited. At this stage for comparison control explants were subjected to similar treatment. However, for later growth, medium containing only 2,4-D was used. Resistance of these calli was studied at 1% level of NaCl as described above.

Haploid callus

- → Subjected to selection pressure on NaCl medium → One resistant line selected from four growing sectors → Subcultured with NaCl as well as without it → Line retains resistance with or without selection pressure → Plantlets redifferentiated on media with and without NaCl
  ↓
  Callusing inducible again on NaCl medium
  ↓
  Subcultures show that degree of resistance remains unaltered
- → In controls, from normal cells, most cells die and calli necrosed on NaCl medium

Fig. 1. Simplified scheme of major procedural steps in selecting salt-resistant line in *Datura innoxia*

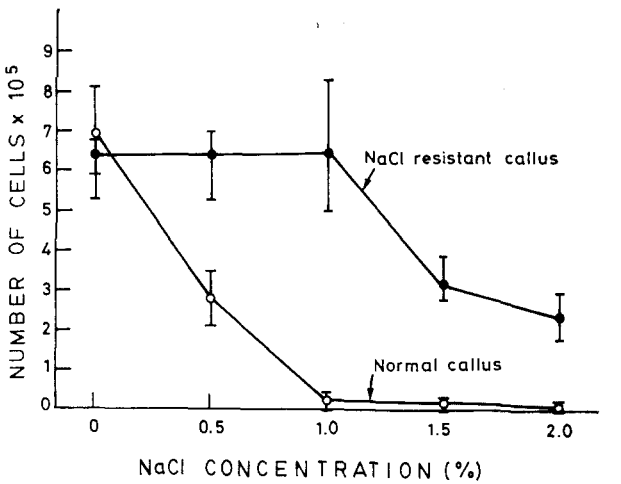

Fig. 2. The effect of NaCl on increase in number of cells of NaCl resistant and normal callus after one month of growth. Data represent mean values of 20 replicates (10 replicates in one experiment) and vertical bars represent absolute minimal and maximal variations in values

3. Results and Discussion

A cell line of *Datura innoxia* resistant to NaCl was selected from the experiment initiated in June, 1977. This cell line was selected on subjecting to selection pressure a total of 1,200 mg callus (*i.e.*, ca. 8.46×10^5 cells, 24 cultures each initially with 50 mg callus) on a medium containing 1% NaCl.

Growth of normal cells is inhibited severely by NaCl (Fig. 2) and almost no growth was observed at NaCl concentrations 1% and above. By contrast, the resistant cell line grows well and its growth response at 1% NaCl was comparable to that of normal callus on a medium without NaCl (Fig. 2). However, the growth of the resistant cell line was inhibited at 1.5 and 2.0% NaCl, but as much as about 35% of growth—of the maximal attained by normal callus growing on medium without NaCl—could be obtained from resistant cell line even at 2% level of NaCl. This line is, therefore, apparently more resistant to NaCl than those of *N. tabacum* (NABORS *et al.* 1975) and *Citrus sinensis* (KOCHBA *et al.* 1978), and almost identical in behaviour to those selected in *N. sylvestris* (ZENK 1974, DIX and STREET 1975) and *Capsicum annuum* (DIX and STREET 1975).

The cell line selected by us retained the same level of resistance even when cultured without NaCl for one month (detailed data not given). Plantlets regenerated from resistant cell line on media with and without NaCl. However, for a proper development these plantlets were taken through three subcultures during a period of about five months. Plantlets could also be produced from normal callus on the medium without NaCl—but none developed from such callus on NaCl containing medium.

To observe the stability of resistance in the long-term, callus was reinitiated from the plantlets differentiated on medium with or without NaCl. The capacity of plantlets, developed on the medium without NaCl in a period of five months, to form callus and sustain its growth in the *presence* of NaCl shows that resistance could be retained even in the absence of selection pressure for a long period. On the other hand, explants from plantlets differentiated from normal callus, did not produce callus in the presence of NaCl.

The callus developed from plantlets was further subcultured and tested for quantitative assessment of the degree of resistance against NaCl at 1% concentration. Callus from plantlets differentiated on NaCl containing medium as well as with no NaCl retained the trait of resistance for NaCl (Fig. 3). The growth of callus fully matched that of control, *i.e.*, of normal callus on medium without NaCl.

In conclusion, our experiments show that a salt-resistant cell line could be selected in *D. innoxia* by tissue culture method and that cells of plantlets developed from this resistant line have retained this character—now for almost

3 years. Recently, NABORS *et al.* (1980) have also reported regeneration of salt-tolerant plants in tissue cultures of tobacco. However, it remains for future to extend these studies to understand the physiological basis of resistance and its inheritance via gametes.

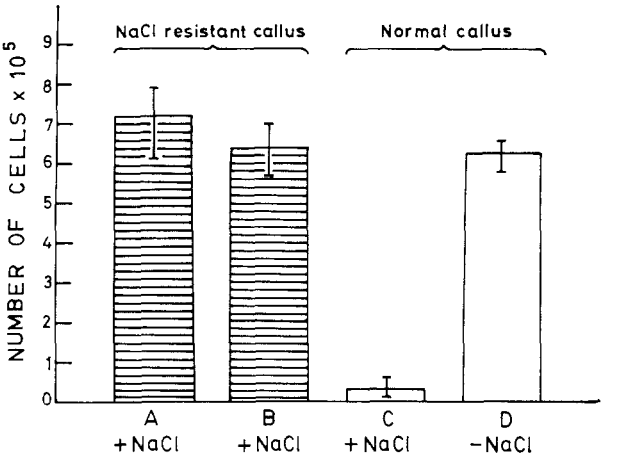

Fig. 3. Increase in number of cells after one month of growth. Callus pieces inoculated on 1% NaCl containing medium were raised from stem explants of plantlets regenerated from NaCl resistant line on NaCl containing medium (*A*) and on medium without NaCl (*B*). For comparison, normal callus was similarly grown also on media with NaCl (*C*) and without it (*D*). Histograms represent mean values of 20 replicates (10 replicates in one experiment) and vertical bars represent absolute maximal and minimal variations in values

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