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ENVIRONMENTAL STRESS RESISTANCE. SELECTION IN PLANT CELL CULTURES

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INTRODUCTION

The selection of variant lines in plant cell cultures has expanded to such an extent that it is no longer easy to review the subject in its entirety. The most recent and comprehensive review[1] covers the variant cell lines which had been described by the beginning of 1979.

The purpose of this report is to look at the progress in the selection of cell lines resistant to various environmental stresses. The rationale behind the selection of this kind of variant is generally practical, the objective being to obtain cultivars of crop plants with a greater tolerance to adverse climatic or soil conditions. A further potential value of resistant cell lines for studies on the nature of stress sensitivity and resistance will also be emphasised here.

Cell lines resistant to several different stresses have been described and these are considered in the following sections.

1. LOW TEMPERATURE STRESS

Steponkus[2] first reported attempts to use callus cultures of *Hedera helix* for the selection of low temperature resistant lines. Although cell lines were obtained which survived the selection procedure, none were found with a stable enhanced resistance to freezing temperatures (Steponkus, personal communication).

Dix and Street[3] obtained cell lines of *Nicotiana sylvestris* and *Capsicum annuum* with enhanced resistance to chilling temperatures. Lines could be isolated with or without prior EMS mutagenesis and were stable in culture. In both species two distinct levels of resistance were found. Fertile plants could only be regenerated from three of the cell lines of *N. sylvestris* with a lower level of resistance, and callus derived from the progeny of selfed plants was found to be sensitive[4]. Respiratory studies using isolated mito-

chondria[3] revealed differences between resistant and sensitive lines of *C. annuum* comparable to those previously found between diverse species varying in low temperature sensitivity.

Variant lines of this type are selected on the basis of survival after exposure of cell aggregates or callus pieces to a chilling treatment. Possible complications of this procedure, compared to the more conventional method of direct selection, requiring growth in the presence of an antimetabolite, have been recently considered[5].

There are recent and current investigations aimed at applying the same sort of procedure to obtain chilling resistant cell lines of several different species, including *Picea excelsia*[6], *Lycopersicon esculentum*[7], and *Oryza sativa* (Le thi Xuan, personal communication).

2. HIGH SALT STRESS

High soil salinity is a serious agricultural problem, particularly in artificially irrigated regions, and the possibility of breeding for salt resistance through selection in plant cell cultures is currently attracting considerable interest. NaCl resistant cell lines have been reported for *Nicotiana sylvestris*[8,9], *N. tabacum*[10], *Capsicum annuum*[9], and *Citrus sinensis*[11], and preliminary work on other species is being carried out in a number of laboratories.

To date there are no reports on the sexual transmission of the resistant phenotype, and there has been little characterisation of the cell lines.

Here, I am able to relate some recent results obtained with a NaCl resistant cell line of *N. sylvestris*. The line, designated NR120 was isolated in the same way as, and has similar morphological properties to, those described earlier[9]. Plant regeneration was, however, possible from NR120, after transfer of cell aggregates to RMO medium[12]. Callus was initiated from the leaves of regenerated plants, and tested for resistance to NaCl on RMP medium[13].

Callus from plants regenerated after only two passages selection in 2 % NaCl showed limited sectorial growth, if any, suggesting continuing segregation for salt resistance. After six passages selection, however, most of the regenerated plants gave callus with a

comparable level of resistance to NR120 callus.

To date, four of these plants have flowered. Two of them (NR120, 6.I and 6.N) were male-sterile and self-sterile, although some seeds were obtained after pollination by wild-type *N. sylvestris*. The other two (6.A and 6.L) were fully fertile (Maliga, personal communication). Sexual transmission of NaCl resistance is currently under investigation in callus derived from the seedling progeny of the regenerated plants.

The NR120 cell line has been used to study the possible basis of salt resistance (Dix, Ferke and Toth, under preparation). X-ray microanalytical investigations, supported by atom absorption data, have shown that Na^+ and Cl^- ions can be taken up equally well by resistant and sensitive cells and so far have provided no evidence for accumulation, or sequestering, of the toxic ions in a particular subcellular compartment, as the basis of salt resistance in *N. sylvestris* cell cultures.

3. METAL TOXICITY

Cell lines of *Lycopersicon esculentum* resistant to aluminium[14], and of *Petunia hybrida* resistant to mercury[15], have been described. In both cases resistant lines take up the toxic ions to the same extent as sensitive lines but no further characterisation has been reported.

Plants have not been regenerated from any of these lines but, in the case of mercury resistance, a genetic basis for resistance is supported by the greatly enhanced frequency of isolation of such lines after chemical mutagenesis[15].

CONCLUDING REMARKS

There are very few reports on the selection of cell lines resistant to environmental stresses. These suggest, however, that it is not too difficult to select such lines, and an expansion of the effort in this area can be anticipated. Where there is evidence that resistance at the cellular level can be translated into resistance in the intact plant, and that resistance is controlled by a small number of genes, there must be a potential for crop improvement using these methods. On the other hand, even in the absence of plant regeneration, and irrespective of whether resis-

tance has a genetic or epigenetic basis, resistant cell lines can provide a useful tool for investigation of the underlying basis of sensitivity and resistance to such stresses as low temperature and high salinity, which cause agricultural problems of global significance.

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