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In Vitro Studies on Salt Tolerance in Rice (*Oryza sativa* L.)

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Introduction

Rice, as other important crops evolving in a glycophytic habitat, is moderately sensitive to salt in the field. The yield potentials of many rice cultivars are largely limited due to the excess of salt in the soil, especially in the coastal regions of rice growing lands of the South and South-East Asia. Attempts to reduce the soil salinity, using mechanical methods (such as reclamation, irrigation and drainage) are not always practical or economical. New varieties, with higher ability to cope with salt stress, are increasingly needed for rice production in the salt affected areas. However, the partially understood complexity of salt tolerance, as observed at a whole plant level, caused the breeding for tolerant plants in this important crop species to be difficult.

Rice represents a group of plants in which the correlation of salt tolerance, compared at cell and whole plant levels, is negative, i.e., the isolated cells are much more tolerant to salt than the whole plant. Cells with increased salt tolerance can be selected from different rice genotypes by *in vitro* culture. However, the tolerant trait selected at cell level through tissue culture was usually not heritable or not stable in the salt stress culture-derived regenerants and their progeny. A critical question is therefore whether the high levels of salt tolerance, as observed at cell level in rice, are dependent on the cycle of certain cell types, along which the tolerant cells are generated.

1. Research strategy

To trace the salt tolerance potential from the cell to the whole plant level, along the ontogenesis, it is important to identify the salt tolerant cell type and characterize the way cells respond to salt during growth and differentiation (5, 6).

2. Materials and methods

Explants used in the experiments (with and without salt supplement) were mature seeds and young inflorescences (*Oryza sativa* L.) from different cultivars and lines of a various japonica-indica genotype cross (1, 2). The *in vitro* studies on salt tolerance included the morphological and biochemical observation and analysis of the differential responses to the continuous and discontinuous NaCl stress regimes of short-term and, especially, long-term cultured rice cells (2, 3, 4, 6, 7).

3. Results and discussion

Results obtained indicate that cultured rice cells may respond variously depending on the intensity of stress present in the culture environment. Embryonic cells have been identified as the salt-tolerant-cell-type in the mix population of different cells. The embryogenic cells grew slowly, generating typical globular structures with high ratio of dry matter, low levels of salt ion accumulation, and produced a specific protein pattern in the 1-D and 2-D gel electrophoretic analysis (6, 8). These cells could give rise to plants through embryogenesis in both salt-free and low salt-containing conditions (3, 4). While high salt stress

inhibits differentiation, the embryogenetic cells can adapt to growing with totipotency retained over sub-cultures in the continuous stress condition (6, 7). The embryogenetic cells tend to differentiate after removal of salt stress from the culture medium. Cells derived from the stress-adapted embryogenetic mass in salt-free conditions are not necessarily of the same type, responsive to regeneration medium and tolerant to salt at the same high level as the previous embryogenetic cells (6). The observed general similarity in responses to salt stress re-exposure of the stress culture-derived non-embryogenetic cells and regenerants and the controlled non-treated cells and plants have led to the hypothesis that tolerance is a natural adaptive potential of the embryogenetic cells and the embryogenetic-cycle dependence is the specific character of salt tolerance in rice (2, 6).

The *in vitro* culture system is useful for stress studies, providing possibilities to generate conditions with more or less similar intensity and regime of stress, in which plants may have evolved in the past and/or to which they are now exposed along the ontogenesis. On the basis of the observations made so far, a general scheme illustrating the effects of salt stress on growth and differentiation of long-term cultured cells in rice, is proposed in *Figure 1* (Binh *et al.*, 1993).

Acknowledgements

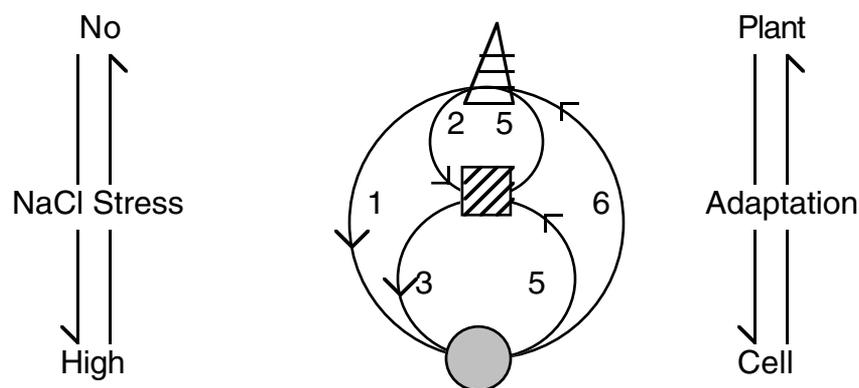
This is a summary of the report that was presented at the session of Rice Biotechnology, FAO Inter-Regional Rice Research Network : Rice Breeding and Biotechnology Workshop, 8-11 February 1994, in Szarvas, Hungary. The long-term research was supported by grants from the Hungarian OMFB and OTKA. The references listed have been limited to only those relating to the summarised "Results and Discussion". On account of a new grant obtained from the UNIDO/ICGB Fellowship Programme, the address of D.Q.B. changed recently and is now : Laboratory of Plant Biotechnology, c/o Department of Genetics and Microbiology, Pavia University, Via Abbiategrasso 207, I-27100 Pavia, Italy.

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Appendix: A general scheme, proposed to illustrate the responses to different salt stress regimes of the long-term cultured cells in rice. Salt stress (left) and the adaptation tendency of the potentially halotolerant embryogenetic cells towards the present-day halosensitive behaviour at whole plant level, along the ontogenesis, of the glycophytically evolved plants (right).

Fig. 1 : Effects of salt stress on growth and differentiation of long term cultured cells in rice



Symbols (-) explants or regenerants, (///) mixed population of different cell types, (®) embryogenetic cells. Callogenesis of explants with (1) or without (2) salt. Selection and growth of embryogenetic cells under high salt stress (3). Unorganized differentiation of the embryogenetic cells in low or no salt stress conditions, resulting in formation of mixed populations of different cell types (4). Organized redifferentiation of cells in the mixed population (through organogenesis or embryogenesis) (5), and plant regeneration (through embryogenesis) from the embryogenetic mass (6) in low or no salt stress conditions.