

FREEZING STRESS RESPONSE IN PROGENIES OF RICE PLANTS REGENERATED FROM CRYOPRESERVED CELLS

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Introduction

Cryopreservation of rice cells and tissues has become progressively useful with the development of biotechnology. To date, this technique can be used to store clones obtained by somaclonal variation or induced mutagenesis (1), as well as transgenic rice lines (2) and transformation-competent rice calli (3):

We previously demonstrated that protoplasts prepared directly from calli are competent for the production of transgenic rice plants (4). Cryopreservation of this type of calli did not affect their plant regeneration competence or the ability of rice cells to integrate and express foreign genes. However, the low proportion of cells surviving cryopreservation suggested that some selection might have taken place, in favor of either specific cell types, epigenetic variants or genotypes. To investigate if freezing stress resistance has been inherited by the progenies of plants derived from these cryopreserved callus lines, we evaluated the effect of freezing temperatures in callus cells and seedlings.

Materials and Methods

Seedlings and callus lines used in these experiments were obtained from seeds of rice plants (cv. Taipei 309) regenerated from different cryopreserved calli and designated hereafter as Cryo 2-plants and Cryo 2-callus lines. Control seedlings and calli established from seeds of the same cultivar were used in all experiments.

To test the freezing resistance of Cryo 2-lines, samples (24 samples/callus line) were placed in 1.5 mL aliquots of solidified culture medium containing the pH indicator chlorophenol red (5, 4). Samples were maintained for either 90 min or 120 min. at -20 °C and then incubated in darkness at 27 °C. Color changes in the culture medium (from red at pH=4.8 to yellow at pH=6) indicating active cell growth were monitored over a period of 7 days. Freezing resistance of cryo 2-plants (20-40 seedlings/seed source/treatment) was evaluated in seedlings (0.25 to 2.5 cm in height) placed in Magenta boxes that contained 100 mL of solidified medium, treated at -14 °C for a period that ranged from 40 min to 2 h. The effect of pre-and post-freezing acclimation was also tested.

Results and Discussion

The response to cryo 2-callus lines to incubation at -20 °C was detectable 2 to 3 days after the freezing treatment based on pH-related color changes in the culture medium. Following incubation at -20 °C for 2 h the number of calli that continue growing decreased to about 40 % in both cryo 2-lines and controls. This ratio increased slightly in most lines following a second freezing treatment, but it generally dropped after the third one. When the incubation at -20 °C was reduced to 90 min., the average resistance of the cryo 2-lines was slightly higher than that of controls.

The survival of seedlings at -14 °C decreased with the length of exposure from a range of 95 % to 65 % in plants treated for 40 min, to a range of 20 % to no survival in plants treated for 2 h. The reduction in growth observed in the surviving seedlings was directly related to the length of exposure to the freezing treatment. This pattern of response was applicable to both cryo 2-plants and controls. Within the cryo-plants, differences in survival did not seem to be related to the seed sources, such as plants regenerated from the same cryopreserved callus or from different batches of cryopreserved calli. Pre-and post-freezing acclimation did not affect the freezing resistance of cryo2-plants, while it generally decreased the survival of the controls. The leaf anatomy and karyotype of seedlings that survived freezing stress is being investigated and will be presented at the meeting.

The general pattern of response to freezing stress, at the seedling and callus level, shown by the progenies of plants regenerated from cryopreserved calli does not differ substantially from that of controls. Therefore, cryopreservation seems to be a reliable way to store transformation competent rice lines.

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