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Abstracts



temperatures (0 - 5°C) the hemolysis values are approximately 3.5 times higher than those for untreated with CPA erythrocytes, and within the temperature range from 5 to 30°C there is a uniform decrease in the hemolysis values from 90 down to 25%.

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P54 CATHEPSIN B-LIKE CYSTEINE PROTEASE APCATHB ADVERSE IMPACTS CELL VIABILITY IN CRYOPRESERVATION

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Cryopreservation-induced cell death is regarded as an important drawback faced by cryobiologists. We isolated a gene, encoding cathepsin B-like cysteine protease from Agapanthus
praecox ssp. orientalis named ApCathB, whose expression pattern had a positive correlation to the occurrence of programmed cell death (PCD) during cryopreservation. The mature ApCathB protein possess in vitro cathepsin B activity and its activity could be inhibited by E-64 and Leupeptin. Overexpression of ApCathB in A. praecox embryogenic callus (OXEC) reduced the survival rate from 45.68% to 18.33% after cryopreservation. Along with the reduced cell viability, the H2O2 and O2·- significantly accumulated in OXEC. The DNA fragment detection showed intensified signal by TUNEL and higher ratio of DNA ladder during OXEC cryopreservation. The cis-elements of TCA-element, ERE, MBS and ARE were responsible for driving the ApCathB expression during cryopreservation treatment. This indicated that the expression of ApCathB was induced by the environment stress of drought and hypoxia and the hormone signals of salicylic acid and ethylene. We analyzed the differentially expression genes (DEGs) of transgenic (OXEC) and non-transgenic (NTEC) embryogenic callus treated with dehydration, dilution and 24 h-recovery to investigate the downstream events effected by ApCathB during cryopreservation. The oxidation reduction process and MAPK cascade were altered in OXEC cryopreservation. Overexpression of ApCathB weakened the antioxidant ability of the cells. In addition, as another partial of stress defense system, the second metabolism and signal transduction pathways were also down-regulated in OXEC. In cryopreservation-induced PCD pathway, ApCathB aggravated the protein proteolytic and inhibiting the anti-apoptosis genes expression. This work verified that ApCathB negatively impact the cell viability through mediate the PCD in plant cryopreservation and provided a novel insight into the mechanism of PCD in cell viability during cryopreservation.

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P55 CRYOPROTECTIVE AGENTS AND LOW TEMPERATURE AFFECT THE ERYTHROCYTE SURFACE MARKER CD44

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Changes in surface markers induced by cryopreservation stresses can be an important sign of cell injuries. This study aims to examine changes in the marker CD44 in erythrocytes under the effect of cryoprotective agents (CPAs) and the impact of cross-linked modification of membrane-cytoskeleton proteins on CD44 over the cell exposure to CPAs, as well as the assessment of consequences of erythrocyte cryopreservation for CD44 alterations. Prolonged exposure of erythrocytes (20 h) with glycerol, Me2SO, sucrose and PEG-1500 led to a reduced CD44 expression and decrease in the CD44+-cell amount in contrast to the short-term exposure, when no changes were found. Extracellular CPAs exhibited a more pronounced effect on erythrocyte CD44 in comparison with the examined endocellular substances. Modification of membrane-cytoskeleton proteins with protein-linking reagent diamide intensified the revealed tendencies. Meanwhile, short-time incubation (1h, 37oC) of frozen -thawed erythrocytes caused a significant decrease in the CD44 expression and in the CD44+-cell amount. These changes were more pronounced in erythrocytes cryopreserved with PEG-1500 than with glycerol. Evidently, time discrepancies in the manifestation of the CD44 changes in erythrocytes exposed to CPAs and cryopreserved ones specify the different processes affecting the cell stability. The changes revealed during cell exposure to CPAs may attest subtle adjustments in protein-protein interactions in erythrocyte membrane-cytoskeleton complex, which are able to increase the cell resistance. Whereas the CD44 alterations in cryopreserved erythrocytes can attest membrane injuries. Since cryopreserved erythrocytes that survived after CPA removal were found to have similar CD44 characteristics as fresh erythrocytes indicating that changes in erythrocytes covered only part of the cells, which were instable and lysed during the washing process. Thus, changes in surface markers of erythrocyte membranes caused by CPAs and freeze-thawing processes can be important for the assessment of structural and functional cell integrity.

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P56 CONVOLUTIONAL NEURAL NETWORK-BASED CELL SEGMENTATION DURING FREEZING IN CRYOPRESERVATION

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