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Molecular Evaluation of Coffee Seeds under Cryopreservation.

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Coffee seeds have poor longevity and are sensitive to desiccation, which limits their storage in conventional germplasm banks. As an alternative method for conserving seeds of this species, storage at ultra-low temperature using liquid nitrogen has been studied. However, the molecular changes that occur in coffee seeds stored at ultra-low temperature are not yet completely known and this is probably the reason for the low rate of success achieved in cryopreservation of these seeds. Thus, the aim of this study was to evaluate expression of genes related to deterioration of coffee seeds under different desiccation, freezing, and reheating protocols so as to broaden knowledge of the effects of cryopreservation on a molecular level in regard to viability of coffee seeds and assist understanding and interpretation of physiological, biochemical, and molecular results obtained through different methods of cryopreservation. The study was carried out in the Molecular Biology Laboratory of the Centro de Café "Alcides Carvalho" of the Instituto Agronômico de Campinas, Campinas, Brazil. We used seeds from *C. Arábica* L. cv. Catuaí Amarelo subjected to different cryopreservation protocols by Figueiredo (2016) to evaluate gene expression by Real Time PCR of the genes apetala 2, ascorbate peroxidase, catalase, dehydrin, DNA methylase, endo-β-mannanase, esterase, glutathione-S-transferase, apoptosis inhibitor, isocitrate lyase, late abundant protein 2, metallothionein, mannose, peroxidase, superoxide dismutase, and telomerase. Relative gene expression was determined from normalization of expression of the target gene with the reference gene GAPDH, chosen for its low standard deviation and coefficient of variation and high Pearson correlation in analysis, using the software BestKeeper. The data of gene expression were compared to the physiological performance of the seeds. Expression of the target genes varied according to the degree of moisture after drying, the pre-cooling procedure, the freezing procedure, and reheating time. Gene expression, except for ascorbate peroxidase, glutathione-S-transferase, and peroxidase, exhibited a relationship to the physiological quality evaluated. For the genes ascorbate peroxidase and glutathione-S-transferase, the lack of relationship with the results obtained by physiological analysis occurred due to the fact of acting for the most part in the initial phase of oxidative stress and through desiccation, they were therefore not very active in cell protection of already cryopreserved seeds, such as those evaluated in this study.

References

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