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Influence of oxygen deficiency and the role of specific amino acids in cryopreservation of garlic shoot tips

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Abstract:

Background: Garlic has lost its ability to form seeds in the course of its domestication. Therefore, the germplasm storage via cryopreservation is increasingly applied. The progression of the various steps within the cryopreservation procedure is accompanied by declining survival rates of the explants. Much of the recent work on cryo-stress has been focussed on osmotic and cold stress components. However, two decades after invention of garlic cryopreservation, the function of metabolites and oxygen in and around the cryopreserved tissues is still largely obscure.
Methods: In this study, hypoxia was characterized in cryopreservation of garlic with oxygen sensors and amino acid metabolism. Furthermore, malondialdehyde, soluble sugars and ammonium were quantified to demonstrate the influence of cryo-stress in declining survival rates.
Results: To better understand the possible reasons for a reduction in the survival rate at the subsequent steps of cryopreservation, the concentration of amino acids, ammonium, γ-aminobutyric acid (GABA), soluble sugars, malondialdehyde (MDA), and oxygen were measured in garlic shoot tips undergoing cryopreservation. Using microsensors, a very low oxygen concentration (< 0.1 μM) was detected within the central meristem region of the shoot apex. When apices were immersed in cryoprotectant solution, the well-oxygenated peripheral regions (foliage leaf base) became likewise hypoxic within a few minutes, probably resulting from strongly restricted gaseous diffusion.
Conclusion: Tissue level oxygen measurements supported the occurrence of hypoxia while biochemical analysis indicated adaptive responses, in particular the modulation in alanine and glutamate metabolism. The possible role of serine and glycine metabolism during cryopreservation is also discussed.

Keywords: *Allium sativum*, PVS3, hypoxia, cryo-stress