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영양체 유전자원의 작은방울-유리화법에 의한 초저온동결보존 실용화기술개발

김행훈, 이정윤, 노나영, 조규택, 윤문섭, 백형진, 김정곤
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Development of Cryopreservation Protocols through Droplet-vitrification and its Application to Vegetatively Propagated Crop Germplasm

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We developed droplet-vitrification protocol, a combination of droplet-freezing and solution-based vitrification, and applied to germplasm collections of garlic, potato, lily as well as cell lines, including hairy roots, somatic embryos. To establish a garlic cryobank, four Korean garlic field collections at Danyang, Suwon, Mokpo and Namhae were cryopreserved last five years. The protocol applied consisted of preculture for 3-4 days at 10°C on solid MS medium with 0.3M sucrose, loading for 40 min in liquid medium with 35% PVS3, dehydration with PVS3 for 150 min, cooling in 5μl droplets of PVS3 placed on aluminum foil strips by dipping these strips in liquid nitrogen, warming them by plunging the foil strips into pre-heated(40°C) 0.8M sucrose solution for 30s. A total of over 900 accessions of garlic were stored in liquid nitrogen for long-term conservation using unripe inflorescences, cloves or bulbils. Twelve alternative plant vitrification solutions were designed by modifying cryoprotectant concentrations from the original PVS2 and PVS3. The results suggest that PVS2-based vitrification solutions with increased glycerol and sucrose and/or decreased DMSO and EG concentrations can be applied for medium size explants which are tolerant to chemical toxicity and moderately sensitive to osmotic stress. PVS3 and variants can be used widely when samples are heterogeneous, of large size and/or very sensitive to chemical toxicity and tolerant to osmotic stress.

Key words : droplet-vitrification, garlic, unripe infloresences, vitrification solution