

# CROP-SPECIFIC GENE BANK STANDARDS FOR *IN VITRO* CULTURE AND CRYOPRESERVATION

Agreed by the *Allium* Working Group

August 2017

Note: the “FAO Genebank standards for *in vitro* culture and cryopreservation” listed in the first column correspond to Chapter 6, pp. 115-158 in: FAO. 2014. Genebank Standards for Plant Genetic Resources for Food and Agriculture. Rev. ed. Rome. ([www.fao.org/docrep/019/i3704e/i3704e.pdf](http://www.fao.org/docrep/019/i3704e/i3704e.pdf))

FAO Genebank standards for <i>in vitro</i> culture and cryopreservation	Crop-specific genebank standards for <i>in vitro</i> culture and cryopreservation [vegetatively propagated <i>Allium</i> accessions, garlic, shallot, great-headed garlic, and other <i>Allium</i> seed-producing species for conditions where seed set is low] <i>No comment in this column means agreement with FAO standard</i>	Remarks (reasons for deviating from FAO standards)
<b>6.1 Standards for acquisition of germplasm</b>		
6.1.1 All germplasm accessions added to the genebank should be legally acquired, with relevant technical documentation.	√	
6.1.2 All material should be accompanied by at least a minimum of associated data as detailed in the FAO/Bioversity multi-crop passport descriptors.	√	
6.1.3 Only material in good condition and of consistent maturity status should be collected, and the sample size should be large enough to make genebanking a viable proposition.	√	
6.1.4 The material should be transported to the genebank in the shortest possible time and in the best possible conditions.	√	
6.1.5 All incoming material should be treated by a surface disinfectant agent to remove all adherent microorganisms and handled so that its physiological status is not altered, in a designated area for reception.	√	Surface disinfectants should be applied immediately before introduction into <i>in vitro</i> /cryo conditions

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<b>6.2 Standards for testing for non-orthodox behaviour and assessment of water content, vigour and viability</b>		This entire section 6.2 is not applicable to <i>in vitro</i> storage /cryopreservation of garlic, shallot, great-headed garlic and other vegetatively propagated alliums!
6.2.1 The storage category of the seed should be determined immediately by assessing its response to dehydration.	N.A.	
6.2.2 The water content should be determined individually, on separate components of the propagule, and in a sufficient number of plants.	N.A.	
6.2.3 The vigour and viability should be assessed by means of germination tests and in a sufficient number of individuals.	N.A.	
6.2.4 During experimentation, cleaned seed samples should be stored under conditions that do not allow any dehydration or hydration.	N.A.	
<b>6.3 Standards for hydrated storage of recalcitrant seeds</b>		This entire section 6.3 is not applicable to <i>in vitro</i> storage /cryopreservation of garlic, shallot, great-headed garlic and other vegetatively propagated alliums!
6.3.1 Hydrated storage should be carried out under saturated RH conditions, and seeds should be maintained in airtight containers, at the lowest temperature that they will tolerate without damage.	N.A.	
6.3.2 All seeds should be disinfected prior to hydrated storage and infected material should be eliminated.	N.A.	

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6.3.3 Stored seeds must be inspected and sampled periodically to check if any fungal or bacterial contamination has occurred, and whether there has been any decline in water content and/or vigour and viability.	N.A.	
<b>6.4 Standards for <i>in vitro</i> culture and slow growth storage</b>		These methods should only be used in special cases, e.g. transient storage of material to be distributed or exchanged.
6.4.1 Identification of optimal storage conditions for <i>in vitro</i> cultures must be determined according to the species.	√	
6.4.2 Material for <i>in vitro</i> conservation should be maintained as whole plantlets or shoots, or storage organs for species where these are naturally formed.	√	
6.4.3 A regular monitoring system for checking the quality of the <i>in vitro</i> culture in slow-growth storage, and possible contamination, should be in place.	√	
<b>6.5 Standards for cryopreservation</b>		
6.5.1 The explants selected for cryopreservation should be of highest possible quality, and allow onward development after excision and cryopreservation.	√	
6.5.2 Each step in the cryo-protocol should be tested individually and optimized in terms of vigour and viability in retention of explants.	√	
6.5.3 Means should be developed to counteract damaging effects of reactive oxygen species (ROS) at excision and all subsequent manipulations.	√	

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6.5.4 Following retrieval, explants should be disinfected using standard sterile procedures.	N.A.	This standard is NOT applicable to <i>in vitro</i> and cryopreservation of <i>Allium</i> spp.
<b>6.6 Standards for documentation</b>		
6.6.1 Passport data for all accessions should be documented using the FAO/Bioversity multi-crop passport descriptors. In addition, accession information should also include inventory, orders, distribution and data user feedback.	√	
6.6.2 Management data and information generated in the genebank should be recorded in a suitable database, and characterization and evaluation data (C/E data) should be included when recorded.	√	
6.6.3 Data from 6.6.1. and 6.6.2 should be stored and changes updated in an appropriate database system and international data standards adopted.	√	
<b>6.7 Standards for distribution and exchange</b>		
6.7.1 All germplasm should be distributed in compliance with national laws and relevant international conventions.	√	
6.7.2 All samples should be accompanied by a complete set of relevant documents required by the donor and the recipient country.	√	
6.7.3 The supplier and recipient should establish the conditions to transfer the material and should ensure adequate re-establishment of plants from <i>in vitro</i> / cryopreserved material.	√	

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<b>6.8 Standards for security and safety duplication</b>		
6.8.1 A risk management strategy should be implemented and updated as required that addresses physical and biological risks identified in standards including issues such as fire, floods and power failures.	√	
6.8.2 A genebank should follow the local Occupational Safety and Health requirements and protocols. The cryo-section of a genebank should adhere to all safety precautions associated with using LN.	√	
6.8.3 A genebank employs the requisite staff to fulfil all routine responsibilities to ensure that the genebank can acquire, conserve and distribute germplasm.	√	
6.8.4 A safety duplicate sample of every accession should be stored in a geographically distant genebank under best possible conditions.	√	
6.8.5 The safety duplicate sample should be accompanied by relevant documentation.	√	