Broadening the spectrum of usable organ sources for cryopreservation of garlic – An AEGIS project report

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Garlic inflorescence with flower buds and bulbil. Photo: J.Keller, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany

Plant germplasm, which can only be preserved vegetatively, often imposes special difficulties for genebanks because its conservation is a time-consuming and costly exercise. Therefore, any method which improves maintenance efficiency is very welcome. In this respect, the most successful technique seems to be cryopreservation. As it is confined to using the very tiny pieces of the plant containing the meristems, its application has some limitations, but it is exciting to broaden the spectrum of usable tissues and thus expand application.

In recent years, publications from the Korean genebank at Suwon were addressing the use of young inflorescence bases of garlic (Kim et al., 2007). Our curiosity aroused, we decided to apply this new strategy under European conditions. We found partners and successfully submitted a proposal in the frame of the first call of the AEGIS Small Competitive Projects (launched in 2009) called "Cryopreservation of young inflorescence bases in bolting garlic for germplasm storage". Three large garlic collections worked together, the Polish collection at Skierniewice, the Portuguese genebank at Braga and the collection at Gatersleben, Germany.

The method is limited to bolting types of garlic, but these form the major part of its genepool, and in terms of usability for improvement through breeding, formation of flower heads is a pre-requisite in any case. Thus, the target genotypes are by far the most important part of the garlic genepool. Usability of young inflorescences is based on the fact that they are full of meristem tissues, since initials of both flower buds and bulbils are present in much higher numbers than meristems in a bulb would ever be. However, there is a limited time span before the flower heads become too old and thus lose their favourable characteristics.

It was necessary to learn that the differences in the local seasons influenced comparability of the results, but our initial meeting in Gatersleben, Germany, came just in time to avoid any problems. Intensive discussions took place, and a field visit updated the knowledge of the participants. The experiments were then grouped into two categories. Most important was the implementation of a standard experiment, which was conducted with the same three accessions previously exchanged between the collections and the standard protocol. This was based on the conclusions of previous projects of colleagues in the cryopreservation community (Reed et al., 2004). 'Standardisation' is not only a buzzword, it is the reference for comparability between the various laboratories and the basis of mutual confidence in multilateral projects. Based on the standard projects, some variations were organized, e.g. comparison of different basic methods (droplet vitrification on aluminium foil -vs- vitrification in cryoprotectant solution), comparisons of various inflorescence stages, cold storage durations, dehydration times in the cryoprotectant, and comparison of the main cryoprotectants PVS2, PVS3, and PVS4. The participants were allowed to use as much of their own additional material as they wished.

The project resulted in the following findings: in the best combinations of accession/ partner, regrowth rates between 74 and 94 percent were obtained. We found that two of the standard accessions were comparable in all three places, whereas one was heterogeneous resulting in regrowth rates between 12 and 89 percent. This shows that the differences of cryopreservation results between different accessions do not depend on the genotype only. Other components are also important, e.g. growing conditions, plant vigour and personal peculiarities in preparation of the explants. Of the two cryopreservation methods compared, droplet-vitrification was clearly superior over the vitrification method. The results did not differ between early, middle or older inflorescence stages so far selected for the experiments. Dehydration periods and various cryoprotectant mixtures did not show a clear picture, and further tests should be done for further refinement in future.

In conclusion, we recommend using this method for bolting garlic everywhere in European collections. It has the advantage that the material is much cleaner than bulbs dug out of the soil, there is direct access to the material without long pre-culture (as is the case for *in vitro* donor plantlets), and the regenerating explants usually produce many shoots due to the presence of several meristems in the cryopreserved pieces of tissue.

When the curators organize their work schedule well, they can combine cryopreservation of young inflorescences in early summer with that of bulbils in autumn and winter, thus attaining maximum efficiency with this strategy.

References:
Kim et al. 2007. Cryopreservation of garlic germplasm collections using the droplet-vitrification technique. CryoLetters 28: 471-482.
Reed et al. 2004. Evaluation of critical points in technology transfer of cryopreservation protocols to international plant conservation laboratories. CryoLetters 25: 341-352.

The final report of the project can also be downloaded at http://aegis.cgiar.org/index.php?id=4470