Leibniz Institute
of Plant Genetics and Crop Plant Research (IPK)

EURALLIVEG AGRI GEN RES action 050

co-funded by the European Commission
under Council Regulation (EC) N° 870/2004[1]

euralliveg.ipk-gatersleben.de

[1] Council Regulation (EC) No 870/2004 of 24 April 2004 establishing a Community programme on the conservation, characterisation, collection and utilisation of genetic resources in agriculture. Official Journal L 162 , 30/04/2004 P. 0018 - 0028

1. Background

The genus *Allium* is a very diverse botanical taxon with several important crops and a high number of potentially very interesting wild species which are also used either in semi-culture or taken from the wild. Onion, shallot, garlic, leek, chives, Chinese chives, and bunching onion are the most important crops. They are used as vegetable and spices, but their medicinal application is recently strongly increasing. Furthermore, there are many nice ornamental species in this genus. Some of them are so showy that they are well presented in large garden exhibitions everywhere in Europe and elsewhere. As of many cultivated plants and their wild relatives, the future of their diversity is endangered by genetic erosion either through habitat devastation, over-collection or monoculture of some genotypes of the crops. This danger is real for most *Allium* species, but it gets an economic dimension especially in case of vegetatively propagated forms, the maintenance of which is especially expensive. Therefore in the present project, the focus was set on garlic and shallot with the expectation that its benefit will also be valid for the other vegetatively propagated alliums and further on the whole genus.

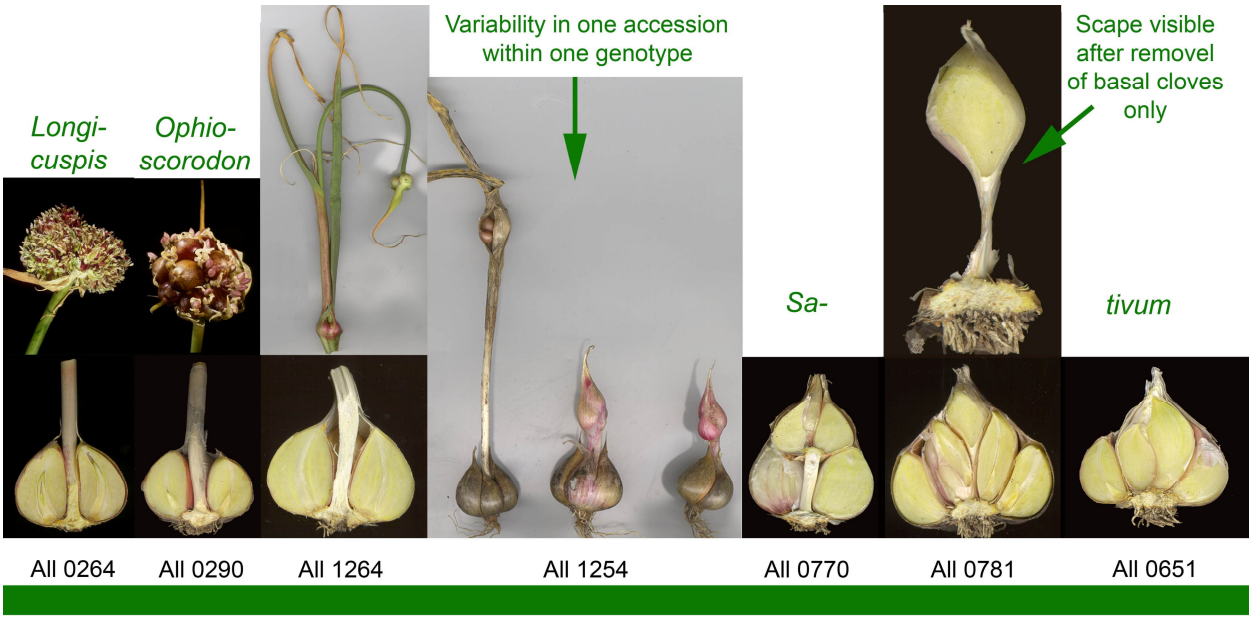
1. The crops and the threats they face

Garlic: Garlic is a multipurpose crop of high importance. It is used as vegetable, spice and in increasing extent, also as medicinal plant (Koch and Lawson, 1996). The production of garlic worldwide is 22.28 million t (2009 – FAOSTAT), only 773,209 t of it in Europe (3.47 %), although Europe maintains the highest diversity of this crop. Garlic production has a long tradition, reports on its culture date back to the antique period (Rabinowich and Currah, 2002). Spreading out from its centre of origin in Central Asia, the introduction into Europe in ancient times led to a very high diversity of this crop, which is caused by the high extent of geographic diversity on this continent and many different cultures and traditions in its populations. This is reflected by the existence of different subgroups in this species, which are termed by many specific folk names in the various countries.

As an example, how the infraspecific grouping of garlic corresponds to its phylogeny, the bolting behaviour of the plants is described here. It is thought that garlic, during the history of its cultivation, lost gradually its generative reproduction strategy by permanent positive selection of the vegetatively most productive plants which are clearly those with smaller or no inflorescence stalk.

The inflorescence itself changed from structures with flowers and many small bulbils to a lower number of larger bulbils accompanied by total loss of flowers. Then, the stalk shortened more and more, ending finally in an inclusion of the inflorescence into the bulb. The bulbils appeared more and more clove-like. The resulting structure is then an irregular compound bulb with no separable

inflorescences at all. One of the intermediate types of this evolutionary line is forming even bulbils in several, more than one, levels of the stalk.
At present, this diversity is extremely endangered by invading low-price garlic produced by some dominating countries. The FAO statistics present these figures clearly. The highest production is in China and India which dominate the world production by 80.64 % and 4.80 %, respectively. Europe is faced by the danger to lose its diversity due to reduction in income from trading the own material (Anonymous, 1998). This is a serious justification to protect and safe Europe's high genetic diversity by most appropriate methods. The danger of a worldwide production of only some types within a crop species, the monoculture, has been experienced in the history already several times as, e.g., in potato and maize. A safe bank of most representative garlic germplasm is an essential tool to counteract this danger.



Infraspecific groups of garlic and their bolting characters.

Shallot:

Shallot, botanically representing the vegetatively propagated part of the species onion, has also high impact as vegetable and spice worldwide. The world production is 3.74 million t, production in Europe 355,824 t corresponding to 9.51 %. Though not so extremely, the endangerment of shallot's germplasm is similar to that of garlic, the market is dominated by such countries like China (23.72 %) and Japan (15.24 % of the world production, respectively - Figures given for 2009 by FAOSTAT for onion [incl. shallots], green). Due to the smaller production scale in comparison to garlic, also the state of research is behind that for garlic. This is the reason for the limitation of this project to characterization only. The well-characterized European shallot Core collection is so far maintained

in de-centralized way by the various partners as a field collection. But it will undergo the AEGIS designation process like it is envisaged for other crops in the AEGIS process as well. Holding a well-structured core collection in permanent maintenance, its introduction into the activities of cryopreservation and virus elimination is well-prepared and easy to implement, as soon as the techniques for shallot will be, in future, at a comparable stage to garlic today.

A part of the garlic core collection cultivated in the fields of the Gatersleben genebank

1.2. Cryopreservation and virus elimination of well-characterized material – a prerequisite of future genebanking

Three aspects are important for maintaining germplasm for future: We must be sure about its identity, the material must be healthy and the material must be kept safely. The project integrated substantial initial steps to build up a core collection to which more and more material may be joined in future.
Characterization is done on various levels. It is not only to describe the material; it is also to exclude redundancy from the various individual collections. Morphological characters are recorded by using descriptors formulated in the IPGRI descriptor list (IPGRI et al., 2001). Comparing of passport data and considering past documents of material exchange between genebanks, a number of 81 duplicates were excluded from the collection. Finally AFLP markers were used to describe the diversity of the core collection. Passport and characterization data, infraspecific classification and images are provided to the public in a specific garlic and shallot database and the EURALLIVEG accession catalogue.

Vegetatively propagated plant collections are usually most endangered by accumulation of viruses. Five viruses and virus groups are present in garlic: onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), garlic common latent virus (GCLV), shallot latent virus (SLV), and the allexi virus group. They are differently harmful, and their elimination is also differently easy. Most important is to free the material from the first two viruses (OYDV and LYSV). Therefore, focus was laid on them. Among the accessions of garlic forming the core collection 24 accessions are free of all five viruses. The development of virus-free plantlets is slow in the first phases. Thus, the main aim was introduction of the method. It was successfully introduced and may be used also in future for new material.
Finally, cryopreservation is the method to store the valuable material (Keller 2002, 2005). For this the shoot tips of garlic, derived from bulbils or *in vitro* plants, were trimmed to small explants of about 1 mm in diameter, dehydrated by osmotically effective and glass-forming substances (cryoprotectants) and placed in tubes containing the cryoprotectant solutions. These tubes were then quickly transferred into liquid nitrogen and stored in cryo-containers. A representative number of explants were rewarmed as control samples shortly after that in order to record the regeneration ability of the respective plant material.

1.3. The cryopreserved genebank collection – the best way to maintain the genetic diversity of garlic and shallot

An integrated system of germplasm safety storage was urgently needed in order to protect the endangered material (Keller and Senula, 2003). Because of the very expensive field culture of vegetatively propagated crops, such a germplasm bank needs to be based on cost-efficient methods as well as high characterization and sanitary standards. At the same time, it connects a certain degree of centralization with a multi-local safety duplication system, which allows the access to the whole collection also after damage, destruction or closure of one storage site. This is provided by the Tripartite Cryopreservation Genebank, which is the main outcome of the present project. This collection is closely embedded into the European germplasm integration policy which is explained in the document of the European AEGIS system, for which *Allium* was one of the model crops in the first period.
The elimination of duplicates is thought for the Tripartite Cryobank only, which will be labelled as AEGIS collection. It will, however, not touch any interests of the partner countries to keep their own germplasm in their own countries for their own interests. However, the well structured and characterized germplasm, which forms the core of Europe's garlic diversity, will also be the core subject for all further protection measures.
Whereas the time limit and the power of the project will allow characterizing the whole germplasm of the participants, it is not the case for cryopreservation and virus elimination, because the time frame for these treatments is longer than the

possible duration of any projects. Therefore, for these methods the most valuable part of the germplasm is envisaged forming a virus-free core-in-core collection. This part will be then the nucleation point for further activities using the management experience derived from this first step and aggregating all the next charges of germplasm to this primary core.

1.4. Exploitation of the genebank material

Due to the above explained different state of the art in garlic and shallot, the exploitation is also on a different level. It will be completely possible in garlic, but it will also be drastically improved in shallot.
In garlic, better exploitation will be possible by a permanent holding of the germplasm in cryopreservation. As resulted from former research, the availability of cryopreserved germplasm is in a similar timeframe like germplasm disseminated in form of bulbils. This ensures access to all germplasm in a feasible manner. The main advantage of cryopreserved material is, that once held in cryopreservation, it will be recovered through *in vitro* culture and can be sent as *in vitro* sample, which facilitates extremely all mailing and transfer procedures, because the material has been separated completely from soil, thus, reducing all endangerments by soil-borne pathogens.
The highest exploitation advantage is, finally, the use of virus-free material from the core collection, because this enables the user to rely on certified material, fully usable without additional cleaning needs. As the project is designed to build up the fundament for future activities in the same direction, it will lay the background for a drastic improvement of the germplasm health and, insofar, its implementation will have impact also in the further germplasm development strategy.

2. Communicating value

The EURALLIVEG project improved collaboration and consolidated the knowledge about the diversity of garlic and shallot in Europe. Through the implementation and further maintenance of the Tripartite Garlic Collection stored in liquid nitrogen a fundament was created which can be enlarged in further time, also by joining of other European collections. The collection will be presented for common use in the EURALLIVEG catalogue and on the image database of the EURALLIVEG Garlic and Shallot Core Collection
http://www.ipk-gatersleben.de/databases/genetic_resources/gscc.
This website, which was implemented by the Bioinformatics and Information Technology Group together with the Genebank Documentation and *In vitro* Storage and Cryopreservation groups of IPK, containing the IPK priority accessions, will include all the material of the EURALLIVEG collection in near future. It will serve as the basic data source for all those that are interested to find out material with special characters for further utilization. A manuscript

about this database has been submitted (Colmsee et al., submitted). Furthermore, the website of the EURALLIVEG project will be maintained for the next 10 years, in which all information on the material and its preservation will be available for all users.
Further publications are in press or will be prepared about the results of the project.
The experience gained in the EURALLIVEG project has been and will be further discussed in the *Allium* Working Group of the Vegetable Network within the European Cooperative Programme of Plant Genetic resources ECPGR. The EURALLIVEG label will be attached to the accessions in the European *Allium* Database.

Cryopreservation tanks of the genebank at IPK (left) and first regeneration stage of an explant after rewarming from cryopreservation (right)


Discussion on correct preparation of explants (left) and training course on *in vitro* plant preculture (right)

3. The Action and the Partners

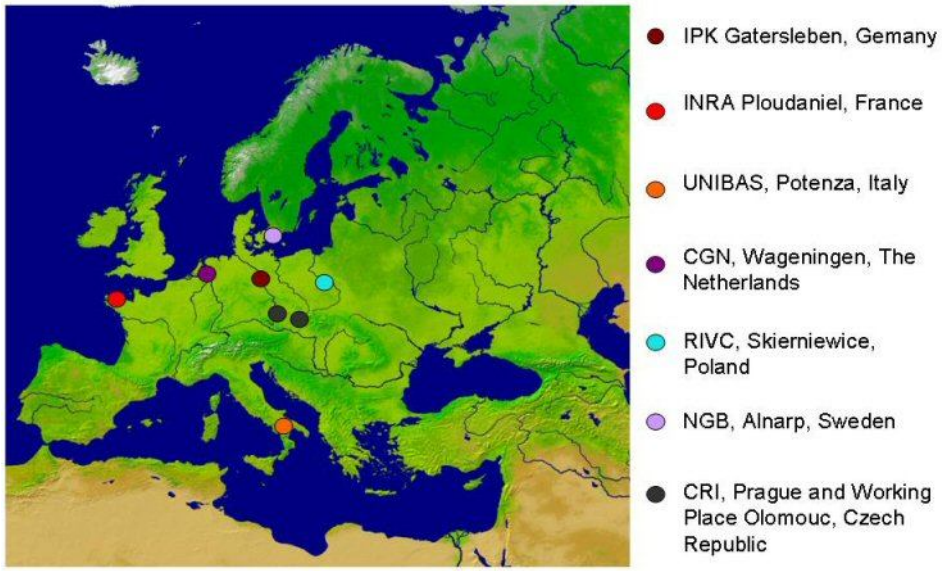
3.1. Action details

EURALLIVEG was awarded 544.500 euros from the EU, towards a total project cost for the GENRES project of 1.089.000 euros. The project started on 1 April 2007. The end-date was 31 March 2011. We, the Leibniz Institute of Plant Genetics and Crop Plant Research Gatersleben, Germany, coordinated the action. Our partners were specialists from organisations located in five EU Member State institutions – in the Czech Republic, Poland, Italy, the Netherlands, France and with the genebank system of the Nordic countries (NordGen).

First meeting of the partners at Gatersleben

The action was implemented in close collaboration and discussed and coordinated in four meetings of all participants, in four smaller and special meetings of work package related groups and three training courses. The resulting cryopreserved garlic collection was safety-duplicated between the cryo-genebanks of Czech Republic, Germany and Poland.

3.2. Partner details



Location of the partner institutions

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4. Links

This section lets you know how to find out more about the “outputs” of the EURALLIVEG project.

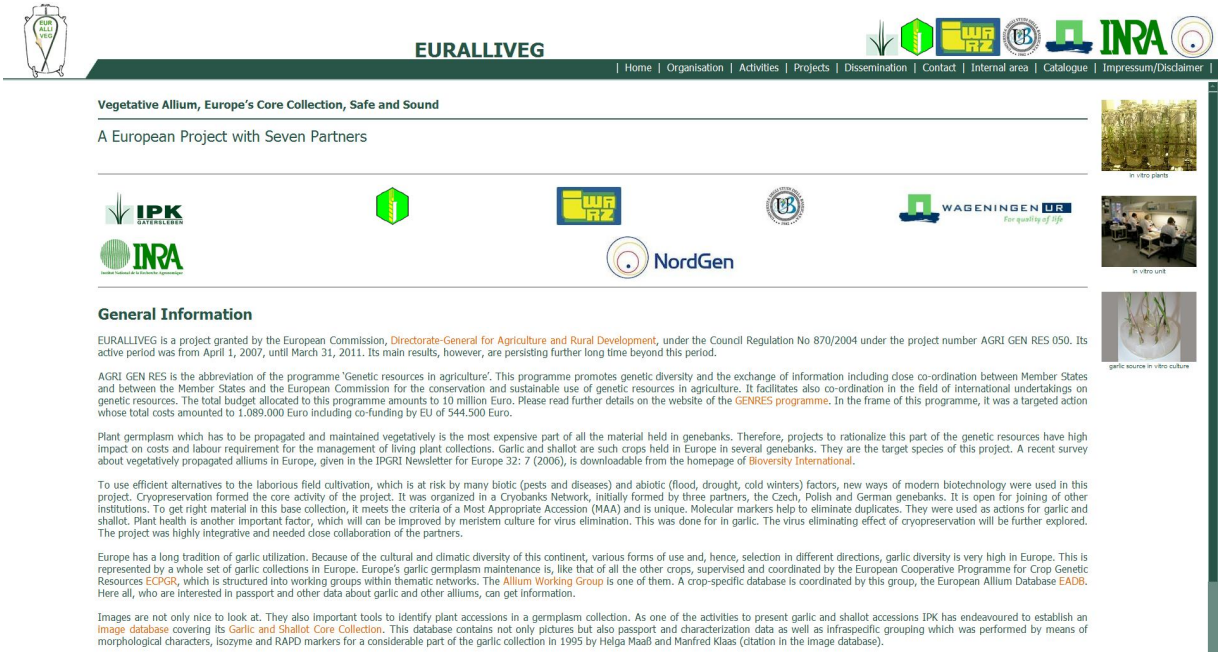
4.1. The genetic resources

If you want to know more about preservation methods of garlic and shallot, we will be glad to hear from you and give you more information. For this, please, contact one of the email addresses given above in the partners' list or look at our websites.

4.2. The database

The main advantage will be for both crops that, with a finally updated and complete database for both crops, the users worldwide, but especially in Europe will have immediately access to the information about the distribution of the desired germplasm within the partners' countries, its passport and characterization data, without getting confused by undesired redundancy. Another product facilitating exploitation is a much better knowledge about the genetic structure of both crops allowing identification of gaps for future

germplasm collecting activities, for research on the crops and for further breeding activities.



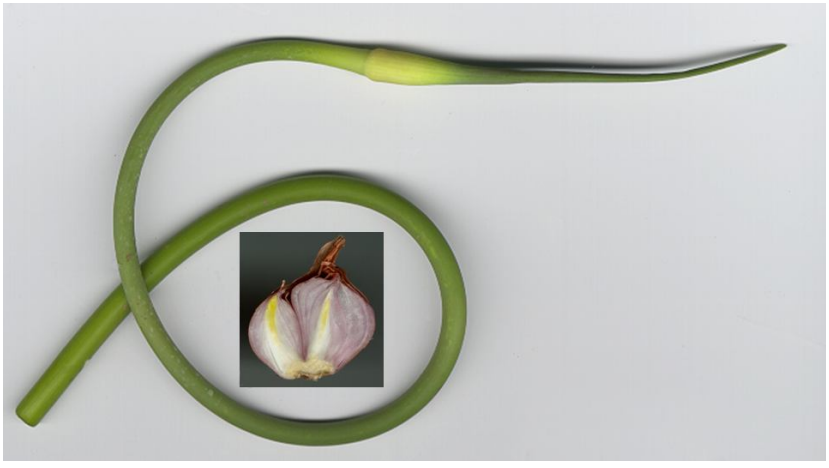
Screenshot of our database

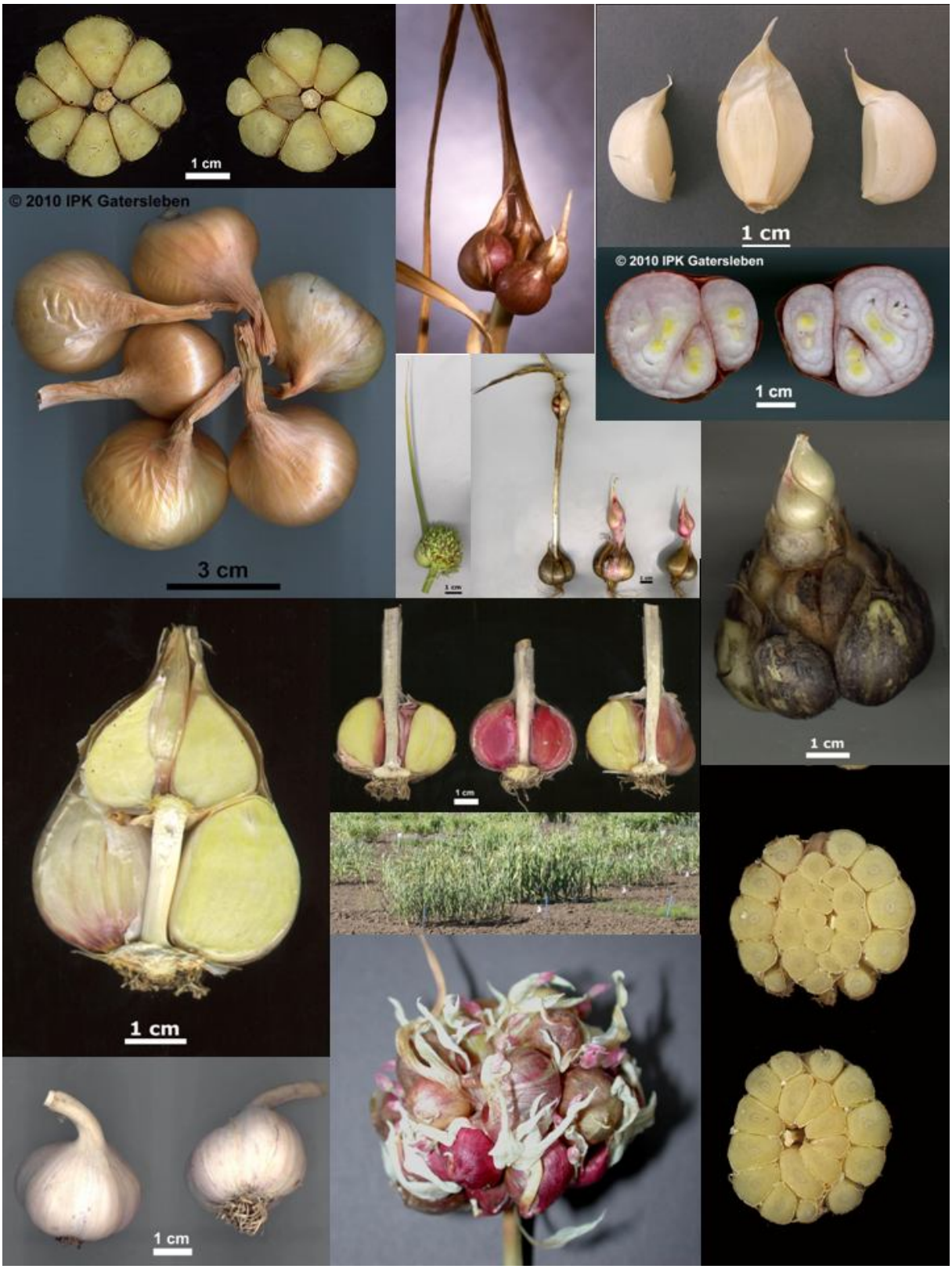
http://euralliveg.ipk-gatersleben.de/
http://www.ipk-gatersleben.de/databases/genetic_resources/gscc

4.3. Publications

We are writing technical, scientific and policy reports and publications. Publications that were already available at the time of writing and links, see the list on the project website
http://euralliveg.ipk-gatersleben.de/dissemination/Relevant-Publications.pdf

Other actions co-funded by the European Commission's Community Programme on the conservation, characterisation, collection and utilisation of genetic resources in agriculture can be found at
http://ec.europa.eu/agriculture/envir/biodiv/genres/index_en.htm


Young inflorescence of bolting garlic and longitudinal cut of violet-flesh shallot



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