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RESEARCH ARTICLE

Comparing costs for different conservation strategies of garlic (*Allium sativum* L.) germplasm in genebanks

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Abstract The maintenance of plant genetic resources in living plant collections (genebanks) causes costs due to employment of staff, usage of buildings, equipment and consumables. Since this is especially challenging in vegetatively propagated material, studies were performed for the case of garlic, which is one of the major vegetatively maintained crops in the genebank of IPK Gatersleben. Data were recorded to compare various scenarios of the main strategies field maintenance and cryopreservation. A spreadsheet tool was developed to be used for cost assessment and for drawing conclusions concerning the most effective way of maintenance. Field culture is cheaper in the short term, whereas after a break-even point cryopreservation becomes the more efficient storage method in the long term. This break-even point depends on the particular scenario, which is determined by various factors such as field and in vitro multiplication rates of various genotypes, presence of bulbils in a part of the genepool, the sample size of the accessions as well as the number of stored accessions in cryopreservation. The comparative discussion is exemplified for a 1-year field rotation versus cryopreservation using either in vitro plantlets or a combination of bulbils and unripe inflorescence bases as organ sources. For the more expensive use of in vitro plants cryopreservation becomes less costly than field culture only after 13 years, whereas this is the case already after 8–9 years when using a combination of bulbils in winter and inflorescence bases in summer.

Keywords *Allium sativum* · Case study · Cryopreservation · Cost factors · Field culture · Long-term storage · Vegetatively propagated germplasm

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Introduction

Considering the increasing speed of genetic erosion within the biodiversity, its preservation is a need more and more requiring investigations and investments in order to protect mankind from devastation and starvation of the major part of its population. In addition to the ethical aspect of protecting the various living

beings, it is a matter of economy how we can organize the protective measures as effectively as possible to save a major part of this biological richness for future generations. This task is especially valid for our cultivated plants for which breeding programmes may benefit from conservation. Genebanks are working on *ex situ* preservation of germplasm. Amongst the various crops, those requiring vegetative maintenance are especially labour- and time-consuming, hence, particularly expensive. Work on rationalization is, therefore, most important in this part of the agricultural diversity.

Garlic (*Allium sativum* L.) is a plant species which lost its fertility during domestication and needs to be propagated by cloves derived from the compound bulbs or by bulbils from the inflorescence, which are formed in the majority of the genotypes. Though production of fertile garlic, providing true seeds, is one of the goals of modern horticultural breeding (Jenderek 2005; Kamenetsky et al. 2005), its extent is still low and so far vegetative propagation will remain the main strategy still for very long time unless forever. Embedded in a very large living *Allium* collection, IPK Gatersleben maintains a high number of garlic accessions, which amounted to 491 at the time of this study (August 2010). This material was assembled in course of collection missions in various parts of the world, in campaigns to secure local material of Germany and by material exchange. Due to intense taxonomical investigations and research on relationships on the interspecific and infraspecific levels of the genus *Allium*, this material became very valuable. Efforts to secure this material in genebanks are, therefore, highly justified.

Four main strategies are followed in genebanks to maintain germplasm. The first way, existing from the beginning of agriculture, is field culture. It may be combined with the second possibility, namely seed storage, where this is possible. In the material of our interest, genebanks were forced to maintain the material permanently in the field until the development of the third option, in vitro storage, and finally of cryopreservation. Permanent field culture bears the risk of accumulation of diseases, mainly viruses, because the seed phase excluding many viruses from the plant, does not exist. In vitro culture was developed in the middle of last century and maintenance on the basis of in vitro storage is feasible in many plants. For *Allium*, however, in vitro storage of germplasm seems not promising in the long term, because of fast weakening and endophyte accumulation in the cultures (Keller 2005; Leifert and Cassells 2001). Thus, micropropagation and several culture steps prior to cryopreservation as well as recovery culture after rewarming until the plantlets are strong enough for transfer into soil are considered the only phases of application of in vitro culture in *Allium*. These phases embed cryopreservation as the ultimate option for long-term storage. Cryopreservation consists of storing small plant organs containing tissue suitable for further development, i.e. shoot tips with meristems, in or above liquid nitrogen (LN) at temperatures of −196 °C (in LN) or maximally −140 °C (above LN). Comprehensive surveys on this technique were given by Benson (1999), Fuller et al. (2004), and Reed (2008). The small size of the storable plant parts requires high labour input for preparation. The further steps are dehydration and cryoprotection. After cryopreservation the explants need to be rewarmed, cryoprotective substances must be removed and then a phase of recovery will lead to plant formation again. Assessment of the method needs to consider that there are three phases of cryopreservation, namely the introduction involving the labour inputs for preparation mainly (including regeneration controls) and the maintenance (storage proper) which consists in refilling of LN storage tanks only. The third phase concerns only such material which will be retrieved for use—the recovery phase which needs again some efforts for plant cultivation.

As cost factors determine feasibility and power of any germplasm preservation, various analyses and assessments were already published (Dulloo et al. 2009; Garming et al. 2009; IPGRI/CIAT 1994; Koo et al. 2004; Li and Pritchard 2009). Cryopreservation was also subject of preliminary economical considerations in Germany and France (Harvengt et al. 2004; Keller 2006; Schäfer-Menuhr et al. 1996).

The present study was performed taking into account the common features of field culture and cryopreservation of garlic as well as its specificities as bulbous plant. A comparison was endeavoured to get better insight in its maintenance under the local conditions of Central Europe. Since there a many more bulbous and tuberous plants which need to be stored vegetatively, this study could be a model case for them. The objectives were (1) to identify the factors causing costs for field culture and cryopreservation of

garlic under the conditions of the genebank of the IPK Gatersleben, (2) to develop a tool to collect data and calculate costs, (3) to assess costs for field conservation and cryopreservation with regard to the different types of costs, and (4) to compare the two strategies in long-term storage approaches.

Materials and methods

Plant material: garlic

Garlic has some special features that influence the conservation in field culture but also cryopreservation. The most important factor is its character as being a bulbous plant. In contrast to herbaceous plants like potato presenting their buds at the tip of shoots, bulbs hide them in the innermost part of their body, which requires higher preparative efforts and, hence, more labour input (Keller et al. 2011). Bulb formation is an adaptive strategy of plants to survive unfavourable conditions such as cold winter periods and dry and hot summer seasons. Consequently the plants follow more or less strictly a seasonal rhythm, which needs special consideration when performing in vitro culture and cryopreservation. Plants need some conditions to be stimulated such as cold treatments but they can also fall into dormancy which may then be difficult to break. Seasonality is also accompanied by limited periods in which the source organs are available. This is a contrast to herbaceous plants, which might be cultivated in greenhouses year-round and provide shoot tips during the whole year. As usual in other crops also, there is a genotypic diversity so that various strategies have to be envisaged because some ways of propagation are not feasible in some parts of the genepool. Thus, e.g. bulbils being a suitable source for cryopreserved explants, are formed in several subtaxa only (Keller and Senula 2001; Maaß and Klaas 1995). In course of the domestication process, focus had been laid on big bulbs. This was accompanied first by loss of fertility and later by gradual reduction of the shoot formation resulting in completely non-bolting types (Etoh and Simon 2002). Examples for these types are shown in Fig. 1. This inability to produce complete shoots of some genotypes may also be accompanied by weaker growth reactions in general as observed in IPK's in vitro collection (data not shown). Thus, for a cost calculation of the complete garlic collection the different biological features of the accessions have to be taken into consideration (see below).

Field culture

Field culture was not only established to maintain the germplasm. It is also the method needed for all the taxonomical investigations conducted in course of the *Allium* research at IPK. Due to the specific tasks field culture is performed in various conditions. The main bulk of garlic (397 accessions) is held in the so-called *Allium* Permanent Garden amongst other species (800 plots), where it is replanted in a 4-year rotation. This "simplification" is possible because of the relatively continental conditions in Central Germany, in which most of the pests and diseases are reduced in the winter season and because of the fertile soil of IPK's locality. All material which does not find place in this garden is planted together with other species in the "Perennial Garden", also for at least 4 years. A special focal part of the germplasm, the "Core Collection" (Senula and Keller 2000), is, however, replanted annually as usually managed in most other genebanks. The Core Collection consists of 54 accessions and the cost calculations shown in this study are exemplified for this type of field conservation, which is the usual field maintenance strategy in most other garlic collections worldwide. Through the year there are various activities needed in the field culture, namely regularly characterizing the plants (e.g. overwintering check), agricultural measures, phytosanitary treatments, harvest and planting. Because of pests and diseases and unfavourable weather conditions, an average annual loss of 1.5 % of the accessions was recorded.

Cryopreservation

For cryopreservation, prior to the storage in liquid nitrogen, there are some steps necessary to prepare the target organ to these conditions. Sources may be shoot tips directly isolated from bulbs or bulbils or young meristematic inflorescence bases or shoot tips isolated from in vitro-grown plantlets. Regardless the more complicated procedure in the latter case, the advantage may be used that the material had passed a virus-cleaning meristem culture prior to cryopreservation. In vitro plants are also the preferred source when material needs to be stored of non-bolting (not shoot-

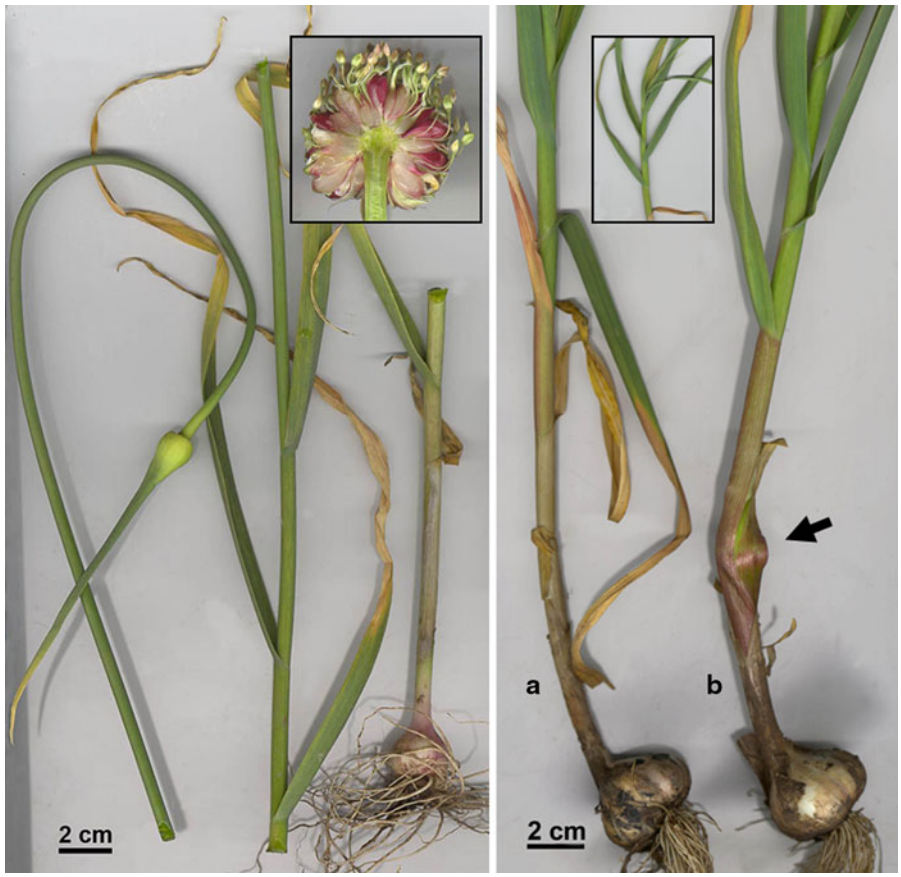

Fig. 1 Examples for different garlic genotypes scanned in June. *Left* accession All 1838 representing the fully bolting type with young inflorescence, *insert* arrangement of flower buds and bulbils in an almost ripe inflorescence of a related genotype later in July; *right* accession All 781, variable genotype with a completely non-bolting (**a**) and a semi-bolting (**b**) representative, upper part of the plant a see insert; the *arrow* marks the place of the inflorescence in the semi-bolting plant

forming) germplasm that is not available in large bulb quantities. In vitro material needs to undergo preculture periods of 1–2 months with low, or preferably, alternating temperatures which increase the vigour of the plantlets (Keller 2005). Once the explants are isolated they need to be pretreated by dehydrating solutions (Loading solution and Plant Vitrification Solution—PVS) in order to reach a state of the tissue in which cell water solidifies as a glass (vitrifies) in course of rapid or ultra-rapid cooling (Keller 2002). A typical cryopreservation protocol is, therefore, a complex procedure consisting of various steps requiring specific treatments and chemicals which influence the cost calculation. The step, which is most labour-consuming and therefore costly, is the preparation of the explants from the source organs. The extirpation of the shoot tips from bulbils needs 3 h for one set of an accession amounting to 150–160 explants. In case of using in vitro plants as source material, their micropropagation to establish the explant numbers needed for the accession, is also costly. More details of the procedures can be found in relevant laboratory manuals (Keller and Senula 2010; Panis 2008).

Cost assessment

The methods applied in this study for cost accounting are based on the models developed by Pardey et al. (1999, 2001) and Koo et al. (2004). Three cost types were distinguished: labour costs (not differentiated into permanent and seasonal employees), fixed costs and variable costs (being dependent on utilisation). An

Excel-based spreadsheet tool was developed to collect the cost items. For each type of costs in each conservation strategy a separate sheet was disposed. All calculations refer to the situation in the genebank of the IPK Gatersleben and the year 2010. Data were collected for gathering an accession into the collection, for maintenance as well as for distribution of an accession to users. For long-term conservation for up to 50 years annual costs were discounted at 4 % and the cumulative present value was calculated for each year (see e.g. Hirth 2005).

In order to evaluate the labour requirement for different steps of the respective procedures, observations and time recordings were undertaken during the routine procedures at the IPK Gatersleben. Moreover, the personnel were asked to journalize their work. In addition all working steps were defined and discussed with the personnel to avoid errors and inconsistencies. The official wages of the gardeners and technical personnel of the IPK compassing the employer's share for social insurances were used to finally calculate the labour costs.

For collecting fixed and variable non-personnel costs, data were provided by the IPK administration or taken from current catalogues of companies dealing with laboratory equipment and consumables. If certain equipment of the IPK was used not only for garlic conservation, distribution keys were developed to calculate the share incurred for garlic conservation. Costs derived from durable means of production were calculated as previously described by Pardey et al. (1999) with an imputed interest of 4 % as well as maintenance and repair costs.

Depreciation was calculated for the different items in a linear way following KTBL (2009). In case of laboratory equipment the expected economic lifetime was calculated based on the experiences of the authors. Variable costs were collected based on the recorded usage of plant protection means, electricity, chemicals and liquid nitrogen, for instance.

Results

Development of the cost calculation tool

The cost calculation tool was created in a way that enables its easy transferability to other situations, be it other wage rates or be it other plant species. Table 1 depicts the assembly of a table sheet to collect data on labour costs for field culture. Column A lists the different steps of the procedure, B the number of employees involved, C the frequency of the respective steps per year, D–F the duration, G effective labour cost per hour, and finally J the annual costs of the respective step. Correspondingly, Tables 2 and 3 illustrate the data collection for capital and variable non-personnel costs, respectively. The Excel file may be made available on request by the corresponding author.

Calculation of costs for field conservation and cryopreservation

The annual costs for field conservation were dominated by the labour costs which accounted for 38.26 € per accession, representing about 81 % of the total costs (Table 4). Among the labour costs two working steps caused the main parts, namely the preparation of the plant material, especially the cleaning of the cloves, and most importantly the manual weeding during the growing season. These labour costs may be compared to much lower capital and variable costs of 3.83 and 5.28 € per accession and year, respectively.

The cost calculations for cryopreservation were much more elaborated and are summarized in a simplified form in Table 5. Again high costs of 186.86 € per accession (52 %) for labour were recorded, mainly caused by establishment and propagation in vitro (62.45 €), preparation of explants for cryopreservation (43.19 €) and media preparation (15.98 €). But cryopreservation also needed considerable input in terms of capital costs (80.98 €) meaning laboratory rooms and equipment as well as variable costs (94.93 €). The latter were governed by costs for energy needed to air-condition and ventilate the laboratories.

The various organ sources for cryopreservation were taken into consideration for calculating the resulting costs in Fig. 2. In vitro plants were shown to cause higher costs for new intakes which were due to higher labour costs of 247.99 €. In vitro propagation is known to be a labour-intensive culture method, and here the elaborative establishment and subculturing are reflecting the main factors. Compared to this, the combined use of bulbils and inflorescences is associated with much lower labour costs of 143.11 €, while

Table 1 Exemplified format of the developed tool for data collection. Labour costs—field culture

(A) *Allium* core collection

A Working steps	B Employees involved	C Frequency/ year	D Duration (min)	E ∑ Accessions worked	F Duration/ accession (min)	G Labour costs/h (€)	H ∑ annual labour costs (€)	I ∑ accessions	J ACA* labour (€)
I. Initiation of field culture									
1. Preliminary work									
a. Preparation of planting plans	1	1	60	54	1.11	21.72	24.13	54	0.45
b. Database updating	1	1	30	54	0.56	21.72	10.86	54	0.20
c. Preparation of plant labels	2	1	15	54	0.28	18.85	9.43	54	0.17
d. Printing of plant labels	1	1	15	54	0.28	16.84	4.21	54	0.08
∑					2.23		48.63		0.90
2. Field preparation									
a. Field fence	3	0.25	107	54	1.98	16.84	22.45	54	0.42
b. Tillage operations	1	1	27	54	0.49	16.84	7.48	54	0.14
c. Measurement of parcels	2	1	60	54	1.11	16.84	33.68	54	0.62
d. Marking plant holes	1	1	30	54	0.56	15.98	7.99	54	0.15
∑					4.14		71.60		1.33
3. Preparation of plant material									
a. Cleaning of bulbs	4	1	270	54	5	15.98	287.64	54	5.33
b. Fungicide treatment of bulbs	4	1	120	54	2.22	15.98	127.84	54	2.37
∑					7.22		415.48		7.70

Table 1 continued

(A) *Allium* core collection

A Working steps	B Employees involved	C Frequency/ year	D Duration (min)	E ∑ Accessions worked	F Duration/ accession (min)	G Labour costs/h (€)	H ∑ annual labour costs (€)	I ∑ accessions	J ACA* labour (€)
4. Planting									
a. Labeling	1	1	60	54	1.1	17.89	17.89	54	0.33
b. Distribution	1	1	30	54	0.56	17.89	8.95	54	0.17
c. Planting	2	1	65	54	1.20	15.98	17.31	54	0.32
∑					2.86		44.15		0.82
∑ *I. Initiation of field culture*					16.45		579.86		10.75

* ACA annual costs per accession

variable and fixed costs remain more or less unaffected. However, bulbils and inflorescences are not available in many genotypes and their availability is restricted to limited time periods during the year. In total, establishment of new accessions was estimated to result in total expenses of approximately 320 € per unit, if bulbils and inflorescences are used as explants, and 430 € starting from in vitro plants.

In addition to explant types, further biological factors influence the costs (Fig. 3). Major effects can be expected from variations in regeneration rates after rewarming amongst all types of explants and from differences in propagation rates in vitro. Low regeneration rates demand for higher numbers of explants to be cryopreserved, while low in vitro propagation rates require more subcultures to achieve the necessary numbers of explants for cryopreservation. These facts were taken into account for in vitro plants by comparing a favourable case with high propagation and regeneration frequencies to an unfavourable case. Again mainly labour costs rose from 194.86 € under favourable conditions up to 357.97 € in the unfavourable case (Fig. 3). Since the distribution of favourable, medium and unfavourable cases depends on the real situation, there is always a local "reality scenario". In the analysis of the given situation, this scenario was composed of 35 % favourable, 35 medium and 30 unfavourable cases (Fig. 2).

For long-term storage, besides the costs for taking new accessions into the collection, the annual costs for maintenance have to be considered. In case of field culture the annual costs are constant over time as given for the core collection case in Table 4, while in the case of cryopreservation they are very low and only caused by the refilling actions of the cryotanks with liquid nitrogen. These maintenance costs for cryopreserved material were calculated to be 20.88 € per year and accession, if 100 accessions are considered, composed of 1.38 € for manual labour and 19.50 € for liquid nitrogen. They decline with increasing numbers of stored accessions and reach a limit at 4.18 € when all 500 accessions are maintained in liquid nitrogen (Fig. 4). Cumulative costs for the different conservation strategies over time showed a steady increase for the field culture compared to high initial costs and more plain curves for two cryopreservation scenarios (Fig. 5). Interestingly, both cryopreservation strategies became more cost efficient in comparison to field culture after 9 and 13 years (break-even points) for

Table 2 Exemplified format of the developed tool for data collection. Capital costs—field culture

a) Facilities	Area (ha)	Imputed lease/ha/year (€)	Annual costs (€)	Portion of costs garlic (%)	Annual costs garlic (€)	ACA* [∑ accessions 583] (€)
Field	1.5	376.00	564.00	100.00	564.00	0.97

Buildings	Area (m^2)	Cost price/m^2 (€)	Service life (years)	Discount rate (decimals) [imputed interest rate 0.04]	Annual costs (€)	Portion of costs garlic (%)	Annual costs garlic (€)	ACA [∑ accessions 583] (€)
Machine shed (size estimated)	130	165.00	50	0.045	960.10	4.0	38.40	0.07
Store room (size estimated)	40	1,506.72	50	0.045	2,697.62	30.0	809.29	1.39
Office	23.5	1,506.72	50	0.045	1,584.85	5.6	88.75	0.15
Working room	16	1,506.72	50	0.045	1,079.05	5.6	60.43	0.10
∑	209.5				6,321.62		996.87	1.71

b) Equipment	Cost price (€)	Service life (years)	Amortization rate/year (€)	Annual costs (€)	Portion of costs garlic (%)	Annual costs garlic (€)	Annual costs/accession [∑ accessions 583] (€)
Four wheel-drive tractor (25 PS)	28,500.00	12	2,375.00	3,110.00	5.0	155.50	0.27
Four-wheel-drive tractor (100 PS)	55,600.00	12	4,633.33	6,090.50	5.0	304.53	0.52
Tractor-mounted sprayer (600 l)	4,200.00	10	420.00	504.00	10.0	50.40	0.09
Tractor-mounted cultivator (2.0 m)	3,100.00	14	221.00	283.00	10.0	28.30	0.05
Mounted plough (4 blades, 140 cm)	12,500.00	14	893.00	1,143.00	5.0	57.15	0.10
Tractor-mounted hole-digger	3,300.00	12	275.00	341.00	10.0	34.10	0.06
∑	107,200.00		8,817.33	11,471.50		629.98	1.09

* ACA annual costs per accession

Table 3 Exemplified format of the developed tool for data collection. Variable costs—field culture

Cost Factors	Annual Consumption		SPU	Annual Costs (€)	% Garlic Costs	Ann. Garlic Costs	ACA *) **)
Energy	**Units**	**Value**	**(€)**	**(€)**	**%**	**(€)**	**(€)**
Electricity – Office	kWh	904	0.124	121.07	6	7.26	0.01
Heating	kWh	2183	0.45	982.58	6	58.95	0.10
∑	**kWh**	**3087**		**1,103.65**		**66.21**	**0.11**
Upkeep and Repairs Buildings	% Replacm.	**1**	**30.13**	**2,395.68**	**6**	**143.74**	**0.25**
Chemicals and Supplies							
Pesticides	-	1	191.83	191.83	100	191.83	0.33
Plant Substrate	m^3	1	50.00	50.00	50	25.00	0.04
Plant Pots (1.5 l)	Piece	100	0.15	15.00	100	15.00	0.03
Fence Posts (Ø 5 cm, 1,75 m)	Piece	87.50	2.50	218.66	60	131.20	0.29
Wire Mesh Fence (1.25 x 697 m)	m	174.25	2.02	352.51	60	211.50	0.47
Stake Labels	Piece	58	2.50	145.00	100	145.00	0.25
Paper Bags (for 1 kg)	Piece	640	0.05	32.00	100	32.00	0.05
Paper Bags (for 2 kg)	Piece	205	0.07	14.35	100	14.35	0.02
Plastic Labels	Piece	580	0.04	23.20	100	23.20	0.04
Plastic Nets	m	20	0.10	2.00	100	2.00	0.003
∑				**1,044.55**		**791.08**	**1.52**

Variable Machinery Costs	Costs for Technical Inspection / Unit (€)		Total Costs / Year (€)	Diesel Fuel Cons. (l/h)	Diesel Fuel Price / l (€)	Diesel Fuel Costs / h (€)	AUY	Ann. Garlic Costs	ACA *) ***)
4-Wheel-Drive Tractor (25 PS)	h	5	9.82	4.30	1.20	5.15	10 h	149.66	2.77
4-Wheel-Drive Tractor (100 PS)	h	6.75	16.55	8.75	1.20	10.47	1 h	27.02	0.50
Tractor-Mounted Sprayer (600 l)	m^3	0.45	0.45				0.46 m^3	0.21	0.004
Tractor-Mounted Cultivator (2m)	ha	4	4				0.8 ha	3.20	0.06
Tractor-Mounted Plough	ha	12	12				0.05 ha	0.60	0.01
Tractor-Mounted Hole-Digger	h	1.5	1.5				2 h	3.00	0.06
∑			**44.32**					**183.69**	**3.40**
∑ Variable Costs per Accession (€)									**5.28**

* *ACA* annual costs per accession, ** [∑ accessions 583], *** [∑ accessions 54], *SPU* sales price/unit incl. 19 % VAT, *AUY* accumulated usage for garlic/year

bulbils/inflorescences and in vitro plants, respectively. Therefore, the high costs for establishing accessions in the cryo-collection are balanced soon due to the low annual costs for maintenance.

Discussion

The thorough analyses performed in course of more than 1 year showed that, despite a certain level of variability, several general features could be extracted, which confirm results of other plant collections (Garming et al. 2009; Harvengt et al. 2004). The main feature is the permanent character of field costs which remain constant over the years in contrast to the two-step character of costs in cryopreservation. Whereas the high costs in the beginning make cryopreservation obviously an expensive endeavour, their low permanent costs once an accession is in storage increase its desirability the longer the material needs to be stored.

Some findings overlay this general situation. More than in field culture, material varies in cryopreservation. This concerns both availability of source organs and vigour of the plants, whereas multiplication rates vary also in the field depending on the structure types of the bulbs. All this causes the need to calculate various scenarios. Another influencing factor is the frequency of requests. Recovery of material from cryopreservation causes some specific costs which do not exist when material is taken from the field. These special costs may turn cryopreservation unattractive for very frequently requested material. In case of potato, this factor induced categorization of the material so that very frequently requested material was recommended not to enter cryopreservation or to be stored in cryopreservation as safety duplicates only (Keller 2006). However, in case of garlic this need is less prominent as the material is generally not so often requested than potato is. The major part, amounting to 67 % of the accessions, was requested only once in 3 years and only one accession each was requested 6 times and 7 times, respectively. Thus, frequency of requests may be neglected in garlic in contrast to potato. Another factor is the difference in costs when in vitro culture is used as source for cryopreservation. In vitro culture prior to cryopreservation increases its costs (Fig. 3). The consequence is the recommendation to use it only in cases where it is ultimately needed. This is when rare material is endangered in the field by some reasons (e.g. susceptibility to diseases) and should be multiplied then in vitro or when meristem culture or cryotherapy need to be performed

Table 4 Annual costs for maintaining one accession in the field collection (core collection) of the IPK Gatersleben

Labour costs	ACA* labour	Capital costs	ACA capital	Variable costs	ACA variable
I. Initiation of field culture		*Facilities*		*Supplies*	
Preliminary work		Land			
a. Preparation planting plans	0.45	Field	0.97		
b. Database updating	0.20	Buildings			
c. Preparation plant labels	0.17	Machine shed (est.)	0.07		
d. Printing of plant labels	0.08	Storage room (est.)	1.39	Energy	
$\sum$	0.90	Office	0.15	Electricity—office	0.01
Field preparation		Working room	0.10	Heating/hot water	0.10
a. Field fence	0.42	$\sum$	2.68	$\sum$	0.11
b. Tillage operations	0.14	Equipment			
c. Measurement of parcels	0.62	Machinery			
d. Marking plant holes	0.15	Small tractor (25 PS)	0.27		
$\sum$	1.33	Big tractor (100 PS)	0.52	Chemicals/field consumables	
Preparation plant material		Mounted sprayer	0.09	Pesticides	0.33
a. Cleaning of bulbs	5.33	Mounted cultivator	0.05	Plant substrate	0.04
b. Fungicide treatment bulbs	2.37	Mounted plough	0.10	Plant pots (1.5 l)	0.03
$\sum$	7.70	Mounted hole-digger	0.06	Fence: posts	0.29
Planting		$\sum$	1.09	Fence: mesh wire	0.47
a. Labeling	0.33	Office equipment		Stake labels	0.25
b. Distribution	0.17	PC	0.012	Paper bags (1 kg)	0.05
c. Planting	0.32	Monitor	0.004	Paper bags (2 kg)	0.02
$\sum$	0.82	Printer	0.004	Plastic labels	0.04
		Furniture	0.010	Plastic nets	0.00
II. Conservation, field culture					
Ratings		$\sum$	0.030	$\sum$	1.52
a. Overwinter survival rating	0.20				
b. Shooting rating	0.20	Tools/working clothes		*Maintenance*	
c. Further ratings	0.30	Spades (ideal)	0.003	Upkeep	
$\sum$	0.70	Secateurs	0.003	Buildings	0.25
Plant protection—spraying	1.04	Knives	0.001	Variable machine costs	
Field work		Hoes	0.002	Tractor 25 PS	2.77
a. Weeding, manually	18.94	Measuring tape (50 m)	0.001	Tractor 100 PS	0.50
b. Weeding, mechanically	1.38	Rainwear	0.006	Mounted sprayer	0.00
c. Replanting (if requested)		Work clothes	0.007	Mounted cultivator	0.06
Direct replanting	0.44	Rubber boots	0.002	Mounted plough	0.01
Greenhouse preculture	0.15	Work shoes	0.006	Hole digger	0.06
$\sum$	21.95	$\sum$	0.031	$\sum$	3.65
Harvest of bulbs/bulbils	4.73				
Storage outside the field	0.13				
$\sum$ Labour costs	38.26	$\sum$ Capital costs	3.83	$\sum$ Variable costs	5.28
Percentage	81		8		11
$\sum$ Total costs	47.37				

* ACA annual costs per accession (all costs in €)

Table 5 Annual costs for establishing one accession (ACA—in €) in cryopreservation at the IPK Gatersleben

Labour costs	ACA labour	Capital costs	ACA capital	Variable costs	ACA variable
Intake of new accessions		*Facilities*		*Operating resources*	
Cleaning of plant material		Buildings		Energy	
Cloves		Laboratory I	14.81	Electricity lab. I	1.42
a. Cleaning	9.08	Laboratory II	5.16	Electricity lab. II	21.82
b. Surface sterilization	2.93	Laboratory III	5.90	Electricity lab. III	1.87
∑	12.01	∑	25.87	Ventilation labs	23.66
Bulbils		*Equipment*		Heating laboratories	2.50
a. Cleaning	9.47	Only used for garlic		∑	51.27
b. Surface sterilization	2.93	Clean bench	5.61	Maintenance and repairs	
∑	12.40	Glass bead sterilizer	0.33	Buildings	11.56
Ø Cleaning	12.20	Stereo microscope	2.58	Lab. equipment	8.80
Establishing garlic in vitro		Cold-light source	0.37	∑	20.36
Cloves		Gas safety burner	0.44		
a. Preparation plant material	15.77	Vortex shaker	0.23	*Chemicals and supplies*	
b. Propagation plant material	44.39	Tissue culture chamber	23.82	Lab. chemicals	
∑	60.16	Cryo watch	0.92		
Bulbils		Cryo tank	5.26	Ethanol (70 %)	1.32
a. Preparation plant material	17.39	∑	39.56	NaOCl solution	1.22
b. Propagation plant material	47.35	Used for all cultures		MS salts, vitamins	1.21
∑	64.74	Autoclave	7.42	Agar–agar	2.02
Ø Establishment	62.45	Lab oven/sterilizer	0.86	D-sucrose	0.50
Preparation of explants for cryo	43.19	Analytical balance	0.80	NAA (auxin)	0.00
Cryopreservation		pH-meter	0.36	2i-P (cytokinin)	0.03
a. Loading phase	5.33	Electric water boiler	0.06	Carbenicillin	0.21
b. PVS3-treatment	2.66	Magnetic heat stirrer	0.26	Glycerol	0.55
c. Explant transfer to LN	2.66	Micro-oven	0.14	∑	7.06
∑	10.65	Glassware washer	1.76		
Transfer of tubes into cryotanks	7.99	Refrigerator/freezer	0.37	Supplies	
Regeneration control cryo-explants		Personal computer	0.51		
a. Warming regeneration contr.	4.44	Monitors	0.15	Cryo color codes	0.50
b. Eval. regeneration control		Label printer	0.07	Cryo tubes, 1.8 ml	5.38
1st evaluation after 14 days	6.66	Laser printer	0.08	Cryo labels	1.97
2nd evaluation after 7–10 weeks	9.99	Flat bed scanner	0.11	Petri dishes	2.72
∑	21.09	Heating bath	0.76	Pipet tips	0.37
		Cryo label printer	0.16	Parafilm	0.04
General, organisation at work		Laboratory furniture	1.48	In vitro labels	0.53
a. Preparation of culture media	15.98	∑	15.35	Aluminum foil	0.55
b. Preparation of cryo-solutions	7.99	Tools and vessels		Filter paper (12 mm)	2.38
c. General preparation/cleaning	7.99	Only used for garlic	0.05	Filter paper (9 mm)	0.51
d. Documentation, database	5.33	Used for all cultures	0.15	Scalpel blades	1.29
∑	37.29	∑	0.20	∑	16.24
∑ Labour costs	186.86	∑ Capital costs	80.98	∑ Variable costs	94.93
Percentage	52		22		26
∑ Total costs	362.77				

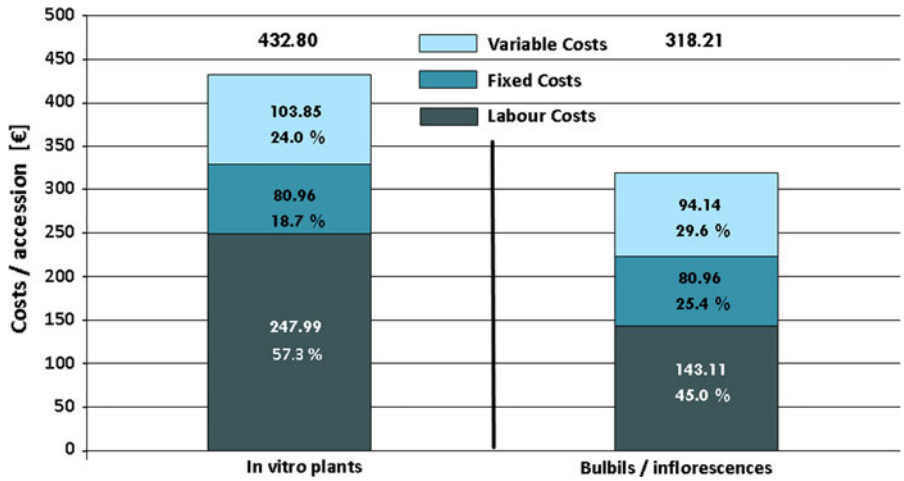

Fig. 2 Comparison of cryopreservation costs per accession for different types of starting material standardised to 100 accessions per year and calculated for the given reality scenario

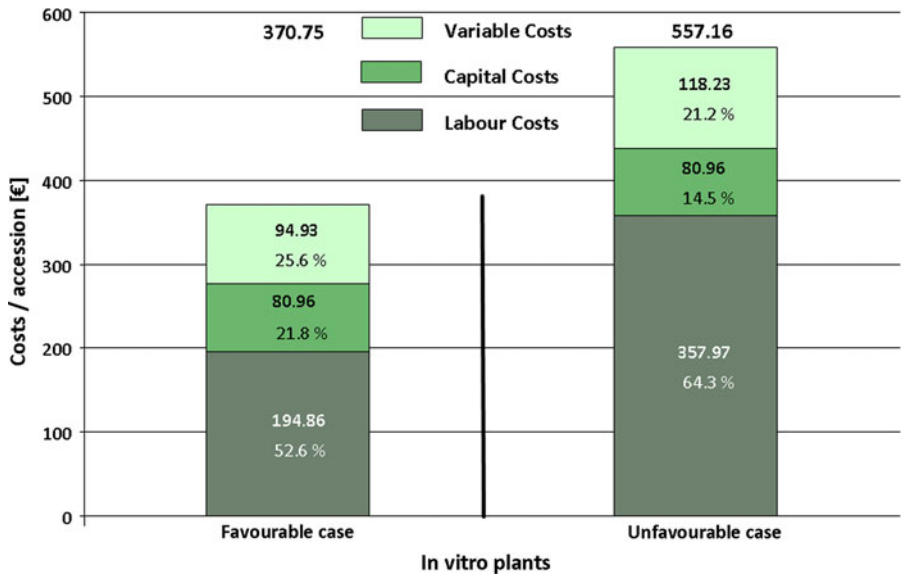

Fig. 3 Comparison of cryopreservation costs per accession using in vitro plants in two different cases standardised to 100 accessions per year. *Favourable case* one subculture for in vitro establishment; *unfavourable case* two subcultures for in vitro establishment, regeneration rate after rewarming lower than 30 % which requires doubling the explant numbers per sample

prior to cryopreservation in order to free the material from viruses first. In other cases, the multiplication phase should be shifted to the field in case of non-bolting material, where no bulbils are available. In case of bolting material, combination of using bulbils in winter with cryopreserving material from young inflorescences in summer is recommended.

Conclusion

The results of analysing the situation of IPK's garlic conservation principally confirm other published data both for other crops in IPK (potato: Keller 2006) and for analyses performed in other places (Harvengt et al. 2004; Schäfer-Menuhr et al. 1996). All these analyses reveal the dominating effects of the labour costs in the introduction phase. They also show that, in the long term, cryopreservation becomes less costly than field culture. These relations are obviously valid for the general situation, regardless the specifics of the given crops or the given situation. Most analyses are limited to the immediate technical situation and do not cover the surroundings, i.e. fix costs of buildings, interest rates etc. Thus, the present study gives a more complete and comprehensive picture about cryopreservation than most other analyses and offers the tool to be adapted to other crops and conditions.

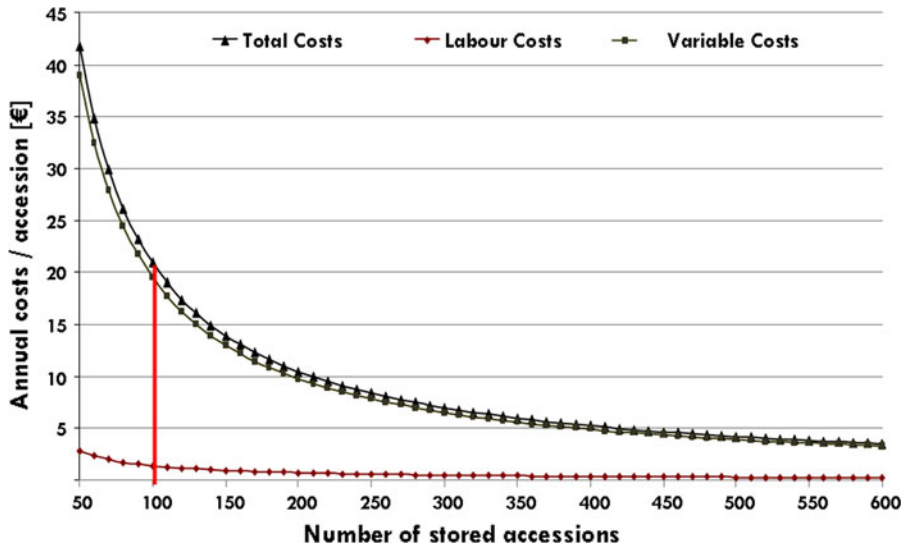

Fig. 4 Annual maintenance costs per accession in cryopreservation in dependence on the number of stored accessions (with 100 explants per accession). The *vertical bar* inside the diagram marks the case of 100 stored accessions

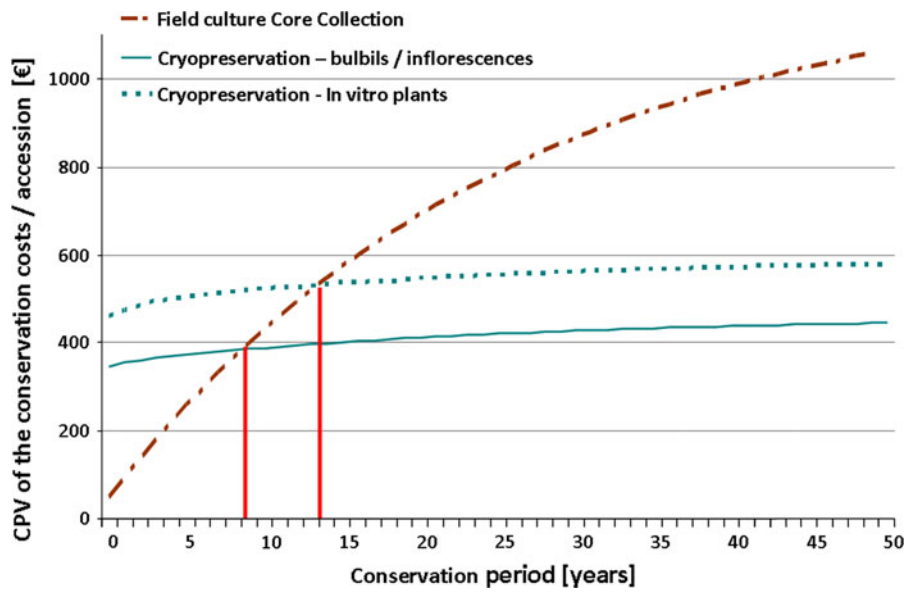

Fig. 5 Example for three cases of cumulative present values (CPV) of the conservation costs for one accession over a time of 50 years comparing field culture in the core collection and two cryopreservation cases. The *vertical bars* inside the diagram mark the break-even points

As usual, improved methods contribute to economizing on the most expensive factor. However, improvement in the field culture may already have reached a point where drastic reduction will not be attainable any more. However, cryopreservation is still a developing method. Thus, considerable improvement may be expected in future, when more insight will be obtained into the processes of oxidative stress and its counteractions by antioxidants, when new methods to cool the material will be developed, as has been reached by adoption of vitrification methods already. The better the expected regeneration rates can be assumed the lower the number of explants may be that need to be stored (Dussert et al. 2003), the lower the labour input is needed finally. The same is true for each kind of recovery measures after rewarming of a sample when needed. This gives cryopreservation an optimistic perspective which should encourage implementing further input of funds which will pay off by lower management costs in the long term.

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