

13 June 2011

*Improving lives
through
biodiversity
research*

Dr. L. Visser
Centre for Genetic Resources, The Netherlands (CGN)
P.O.Box 16
6700 AA Wageningen
The Netherlands

Dear Dr Visser,

Bioversity International¹ wishes to enter into an agreement with Centre for Genetic Resources, The Netherlands (CGN), to undertake activities in relation to the "Identification old potato clones having unreliable variety name by means of fingerprinting using microsatellite (SSR) markers to assist in setting up the AEGIS collection for potato cultivators", one of the ECPGR Competitive AEGIS Grant Scheme project proposals of the Second Call, that has been awarded, on the terms and conditions set out below.

Bioversity International hosts the Secretariat of the European Cooperative Programme for Plant Genetic Resources (ECPGR), coordinates its activities, and has the capacity to enter into legal contracts on behalf of ECPGR.

1. Scope of Work

The work to be undertaken is described in the Project document annexed to this agreement (Annex A).
As decided by the AEGIS Advisory Committee, you are expected to implement the recommendations made by this Committee as indicated on the evaluation of the proposals (see Annex B).

2. Timing

This agreement covers the period from the date of signature of the agreement for a period of 12 months.

3. Specified Personnel

CGN should ensure that the following work, namely the "Identification old potato clones having unreliable variety name by means of fingerprinting using microsatellite (SSR) markers to assist in setting up the AEGIS collection for potato cultivators" is undertaken by Mr Roel Hoekstra.

¹ With effect from 1 December 2006, IPGRI and INIBAP operate under the name "Bioversity International", Bioversity for short.



4. General Reporting

- 4.1 Bioversity International requires reports as set out in 4.4 below. The purpose of the reports is to place on record in a complete, useful and durable form the outcome of this agreement
- 4.2 All reports should include a fact sheet according to the format given in **Attachment A**.
- 4.3 Reports should be written in a clear, simple style and in English. A copy of the Bioversity International Style Guide is available on request.
- 4.4 The final report should be approximately 10 pages long and prepared under the following headings:
Introduction (States the reasons for and objectives of the project. Introduces the activities covered in the report.); Materials and Methods; Results; Recommendations; Bibliography; Attachments (e.g. glossary, maps, tables, illustrations).
A progress report should be submitted at the end of the sixth month after signature of the contract. This report will contain a brief description of the progress and any constraints encountered/foreseen as well as any proposed deviations from the original workplan.
- 4.5 Three copies of the final report, plus a readable wordprocessing file (preferably MS Word file or if unavailable a PDF file) should be submitted to Bioversity International within one month after the end of the contract.

5. Budget and Financial Reporting

- 5.1 Bioversity International has approved a budget totaling **Euro 26,800.00**. Details of the budget are set out in the annexed financial schedules (**Attachment B**) and initialed by the parties for the purpose of identification.
- 5.2 CGN will have the responsibility to disburse directly from the above mentioned amount all payments to the project partners as per the project proposal (see Annex A). Bioversity International will not establish agreements with these partners and will not disburse any payments to these sub-contractors.
- 5.3 CGN agrees to provide Bioversity International with financial statements certifying that the funds have been spent for the agreed purposes and itemized according to the budget headings and with the frequency set out in the annexed financial schedules.
- 5.3 CGN shall keep books, records and original vouchers for up to five years following the date of the completion specified in the LOA.
- 5.4 CGN agrees at all times for Bioversity International representatives to inspect the books and documents kept in connection with the LOA and visit the work site. It shall also supply all information requested by Bioversity International representatives.



6. Payments

- 6.1 Bioversity International agrees to make payments for the work defined in the Scope of Work as set out in the annexed financial schedules.
- 6.2 Bioversity International will not make the prescribed payments unless the required progress and financial reports have been received.
- 6.3 Any unspent funds remaining upon completion of the work will be returned to Bioversity International.

7. Insurance

- 7.1 Bioversity International declines every form of responsibility for actions, claims, demands, costs and expenses which may arise from or be a consequence of any unlawful or negligent act or omission of CGN or its employees or agents in carrying out the work described in the Scope of Work.
- 7.2 Therefore when deemed necessary, CGN should take out appropriate insurance cover for all staff and/or activities financed through this agreement, such as, but not limited to: health, life, accidents, long term disability, workers compensation, travel, public liability etc. The decision whether or not such insurances are required, rests entirely with CGN.

8. Bioversity International Coordinator

- 8.1 The person holding, occupying or performing the duties of AEGIS Coordinator, currently Jan Engels, shall have responsibility for supervision of the Agreement on behalf of Bioversity International and will have authority to issue and receive any written notification under the Agreement.

9. Terms and Conditions of Agreement

- 9.1 The attached terms and conditions marked **Attachment C** and entitled "GENERAL CONDITIONS FOR AGREEMENTS" shall form part of this Agreement.

10. Intellectual properties

- 10.1 All rights, titles and interests in intellectual property based on Agreement Material (including copyright, patent, trade secret, trademark rights and database rights) produced by Bioversity International or its personnel in conjunction with CGN or its personnel will be assigned jointly to Bioversity International and CGN.
- 10.2 Bioversity International and CGN agree to grant one another, non-exclusive, royalty-free licenses to use, reproduce and distribute agreement material without any need to obtain permission from one another for those purposes. Bioversity International and CGN further agree to grant one another the right to grant sub-licenses to their project partners. Both parties agree to make reasonable efforts to ensure that third parties publicly recognize the authors and publishers of agreement materials and the joint institutional support of Bioversity International and CGN in their creation.

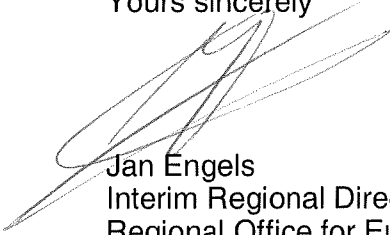


- 10.3 Intellectual property based on work *outside* this Agreement shall remain with each party, and no rights thereto are provided in this Agreement.
- 10.4 Any publications or other outputs resulting from activities carried out under this contract/agreement will be done in consultation with Bioversity International and will include one or more Bioversity scientists as co-author(s), following standard scientific practice.

11. Acceptance

A duplicate of this letter is enclosed with an endorsement that provides for notification of acceptance. If you agree to provide the work specified in the Scope of Work as set out and attached to this letter your acceptance must be notified by signing, dating, and returning the enclosed duplicate letter to Bioversity International. Receipt by Bioversity International of acceptance in writing of these terms and conditions will constitute the entire agreement for the provision of the work described in the Scope of Work.

Yours sincerely



Jan Engels
Interim Regional Director
Regional Office for Europe



(endorsement)

Centre for Genetic Resources, The Netherlands (CGN) agrees to provide the work described in the above letter in accordance with the terms and conditions set out in and attached to this letter.

For and on behalf of Centre for Genetic Resources, The Netherlands (CGN)



Dated this Mon day of 20/6 /..... 2011

Name: dr Bert Visser

Title: Director Centre for Genetic Resources, the Netherlands



BIOVERSITY INTERNATIONAL REPORT FACT SHEET
Attachment A

Library Report No. (Library use only)	
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All required sections of this Fact Sheet are **completed by the Author of the Commissioned Organization or Contractor**. Once completed, this Fact Sheet should be attached to the front of the report.

FULL TITLE of REPORT/PROJECT	Identification old potato clones having unreliable variety name by means of fingerprinting using microsatellite (SSR) markers to assist in setting up the AEGIS collection for potato cultivators	
AUTHOR of Commissioned Organization or Contractor (Name and title of person)		
NAME/ADDRESS of Commissioned Organization or Contractor	Centre for Genetic Resources, The Netherlands (CGN)	
DATE REPORT SUBMITTED		
TYPE OF REPORT i.e. progress/final		
PREVIOUS REPORTS (Please fill in dates and add more lines if necessary) <i>To be completed by BIOVERSITY INTERNATIONAL Coordinator</i>	1 st Progress Report	Date: <i>(within 6th month after start of contract)</i>
	Final Report	Date: <i>(within one month after end of contract)</i>
BIOVERSITY INTERNATIONAL LETTER OF AGREEMENT <i>To be completed by BIOVERSITY INTERNATIONAL Coordinator</i>	11/042	
BIOVERSITY INTERNATIONAL PROJECT CODE <i>To be completed by BIOVERSITY INTERNATIONAL Coordinator</i>	7211EC-F09001	
BIOVERSITY INTERNATIONAL CONTACT	J. Engels – AEGIS Coordinator	
ABSTRACT (Minimum 100 words)		
KEYWORDS	Country/Region: Europe Crop(s): Potato Subject: AEGIS collection	

BIOVERSITY INTERNATIONAL
AGREEMENT FINANCIAL SCHEDULES

DETAILS OF COMMISSIONED ORGANIZATION OR CONTRACTOR	
Surname: Visser	First Name: Bert
Title (Dr etc): Dr. ir.	Position: Director CGN
Institute Name: Centre for Genetic Resources the Netherlands (CGN), Wageningen University and Research Centre (WUR)	
Address: P.O. Box 16, 6700 AA Wageningen, The Netherlands	
Telephone: +31-317-480884	
Fax: +31-317-423110	E-Mail: bert.visser@wur.nl

CONTACT SCIENTIST (if different from above) FOR DETAILED PROJECT INFORMATION	
Surname Hoekstra	First Name Roel
Title (Dr etc): ir.	Position curator
Institute Name: Centre for Genetic Resources the Netherlands (CGN), Wageningen University and Research Centre (WUR)	
Address P.O. Box 16, 6700 AA Wageningen, The Netherlands	
Telephone +31-317-480912	Fax +31-317-423110
Email roel.hoekstra@wur.nl	

DATE OF PREPARATION (day/month/year)	
TOTAL COST OF THE AGREEMENT	Euro 26,800.00

TITLE OF PROJECT
Identification of old potato clones having unreliable variety name by means of fingerprinting using microsatellite (SSR) markers to assist in setting up the AEGIS collection for potato cultivators

<u>For BIOVERSITY INTERNATIONAL office use only</u>	
Project/Activity	
Budget Code/Funding	€ 11,000 on 7211EC-F09001; € 15,800 on 7207EC-F09001
LOA Number	11/042
BIOVERSITY INTERNATIONAL Coordinator	L. Maggioni

BUDGET

Item	Euro
Staff time	17,000.00
Laboratory supplies	9,000.00
Curators supplies	800.00
TOTAL	26,800.00

SCHEDULE OF PAYMENTS, FINANCIAL REPORTS AND BANKING INSTRUCTIONS

1. Payments and Financial Reports

Payment Number	Date of Payment	Amount of Payment	Financial Report Dates
Advance payment (80%)	Upon receipt of signed LOA	Euro 21,440.00	
Final Payment	Upon receipt of final financial and technical report	Euro 5,360.00	30 June 2012
TOTAL		Euro 26,800.00	

2. Banking Instructions

a) Currency required	euro
(b) Bank Account Number	30.70.00.427
In the name of	Stichting DLO PPO-PRI
(c) Name of bank	Rabobank Vallei en Rijn
Address of bank	P.O. Box 21, 6710 BA Ede
Swift/BIC Code:	RABONL2U
IBAN Code:	NL20RABO0307000427



GENERAL CONDITIONS FOR AGREEMENTS

1. Interpretation

1.1 In these Conditions:

- (a) With effect from 1 December 2006, IPGRI and INIBAP operate under the name "Bioversity International", Bioversity for short.
- (b) "the Agreement" means the Agreement under which the work described in the Scope of Work is to be provided to Bioversity International including these General Conditions.
- (c) "the Scope of Work" means the work to be performed under the Agreement.
- (d) "the Commissioned Organization or Contractor" means the party who by the Agreement undertakes to provide the work described in the Scope of Work.
- (e) "the agreement material" means all the material brought or required to be brought into existence as part of, or for the purpose of performing the work described in the Scope of Work including, but not limited to, documents, equipment, information and data stored by any means.

1.2 Words importing a gender include the other gender. Words in the singular number include the plural and words in the plural number include the singular.

2. Variation of Agreement

2.1 No agreement or understanding that varies or extends the Agreement (including in particular the Scope of Work) and would result in an increase in the monies payable by, or other liability of Bioversity International shall be legally binding upon either party unless in writing and signed by both parties.

3. Agreement Material

3.1 On the expiration or earlier termination of the Agreement, the Commissioned Organization or Contractor shall deliver to Bioversity International all agreement material.

3.2 The Commissioned Organization or Contractor shall ensure that the agreement material is used, copied, supplied or reproduced only for the purposes of the Agreement.

4. Permission for publication and publicity

4.1 To ensure the quality of Bioversity International associated products, the Commissioned Organization or Contractor, its employees or agents shall not disclose or make public any information or material produced in connection with or by the performance of the work described in the Scope of Work bearing the Bioversity International name or Bioversity International logo without prior approval in writing of Bioversity International. In addition, any publication arising from the work undertaken under the Agreement, the Commissioned Organization or Contractor shall acknowledge Bioversity International's support.

5. Conflict of Interest

5.1 The Commissioned Organization or Contractor warrants that, at the time of entering into the Agreement, no conflict of interest exists or is likely to arise in the performance of its obligations under the Agreement. If, during the terms of the Agreement, a conflict or risk of conflict of interest arises, the Commissioned Organization or Contractor undertakes to notify Bioversity International immediately in writing of that conflict or risk.

6. Currency of Payments

6.1 Payments by Bioversity International or the work described in the Scope of Work shall be made in the currency of the agreed budget. No compensatory adjustments will be made by Bioversity International for changes in rates of exchange to any other currency.

7. Security

7.1 The Commissioned Organization or Contractor shall, when using Bioversity International's premises or facilities, comply with all security and office regulations in effect at those premises or in regard to those facilities, as notified by Bioversity International.

8. Negation of Employment, Partnership and Agency

8.1 The Commissioned Organization or Contractor shall not represent itself, and shall ensure that its employees do not represent themselves, as being employees, partners or agents of Bioversity International.

8.2 The Commissioned Organization or Contractor shall not by virtue of this Agreement be or for any purpose be deemed to be an employee, partner or agent of Bioversity International.

9. General Principles for Genetically Modified Organisms

9.1 The Commissioned Organization or Contractor hereby undertakes to comply with the Guiding Principles for Genetic Modified Organisms (GMOs) as attached to these General Conditions in carrying out the work to be performed under the Agreement.

9.2 Bioversity International shall have the right to audit the work of the Commissioned Organization or Contractor to ensure that the Guiding Principles for GMOs are being complied with and shall have the right to terminate the Agreement without compensation should it find that the Guiding Principles for GMOs are not being fully complied with.

10. Termination of Agreement

10.1 Bioversity International may at any time by written notice to the Commissioned Organization or Contractor, terminate or constrict the Agreement or any part of the Agreement, and upon such notice being given, the Commissioned Organization or Contractor shall cease or reduce work according to the tenor of the notice and shall forthwith immediately do everything possible to mitigate consequential losses to Bioversity International.

10.2 In that event the Commissioned Organization or Contractor may submit a claim for compensation and Bioversity International shall pay to the Commissioned Organization or Contractor such sums as are fair and reasonable in respect of any loss sustained by the Commissioned Organization or Contractor in unavoidable consequence provided that:

- (a) the Commissioned Organization or Contractor shall not be entitled to compensation for loss of prospective profits;
- (b) Bioversity International shall not be liable to pay any sum which, in addition to any amounts paid or due or becoming due to the Commissioned Organization or Contractor under the Agreement, would together exceed the full cost ordinarily payable under the Agreement; and
- (c) The Commissioned Organization or Contractor shall not be entitled to compensation for any sums that the Commissioned Organization or Contractor is able to recover from any insurance.

11. Default

11.1 If the Commissioned Organization or Contractor fails within fourteen days after receipt of written notice to remedy any default in the performance of the following obligations, namely:

- (a) to commence or to proceed at the rate of progress strictly in accordance with the Agreement; or
 - (b) to perform or observe the terms and conditions of the Agreement,
- Bioversity International may, by written notice, terminate the Agreement and recover from the Commissioned Organization or Contractor any loss or damage suffered by Bioversity International.

12. Settlement of Disputes

12.1.1 Without prejudice to the foregoing, any dispute or disagreement arising from the interpretation or execution of the Agreement shall be settled by arbitration as follows:

- (a) both parties will first endeavour to settle any difference amicably by direct dealings;
- (b) if no agreement is reached within sixty days, the matter will be submitted to arbitration upon the application of either party;
- (c) there will be three arbitrators, one to be appointed by Bioversity International, one by the Commissioned Organization or Contractor and the third, as Chair, jointly appointed by both parties;
- (d) in case of disagreement, the third arbitrator will be appointed according to the Rules of the United Nations Commission on International Trade Law (UNCITRAL). The UNCITRAL Rules will apply to any arbitration proceedings, arbitration will take place in a neutral country and the official language of the proceedings will be English.

Proposal for the identification of 500 old potato clones having unreliable variety name by means of fingerprinting using 12 microsatellite (SSR) markers to assist in setting up the AEGIS collection for potato cultivars.

1. Problem statement

The true identity (cultivar name) of potato clones in different collections is not always clear or correct. This is hampering the selection of the Most Appropriate Accessions (MAA's) for the AEGIS collection.

- In particular for old potato cultivars the clone can be mislabelled, as reported by H. Campbell from SASA (Frese & Hoekstra, 2009).
- Some variety names have been used more than once (e.g. Gloria 1921, 1937, 1972) and it is not always known to the curator what the true identity of the clone in his collection is.
- Based on SSR data, K. Dehmer (IPK, Germany) found for old blue/purple fleshed potato varieties that different names may be synonyms for the same clone.

Summary provided by K. Dehmer: a set of 15 SSR markers was applied onto 26 blue fleshed accessions of the IPK Genebank. Only seven different SSR patterns/genotypes were identified. Four unique genotypes were represented by one GLKS accession each, while the other three genotypes were attributed to three duplication groups consisting of thirteen, five and four GLKS accessions, respectively.

2. Justification and rationale

The selection of the Most Appropriate Accessions (MAA's) by the ECPGR Potato Working Group for the AEGIS collection will be based on the passport data provided by the collection holders. Correct data on the identity of the individual clones and knowledge about synonymy are crucial for this process. This project will provide accurate identifications for clones having questionable name labels (of potato germplasm selected from several European collections).

3. Background

In particular clones of presumably old potato cultivars can be mislabelled. This may be caused by incorrect information from the germplasm donor, or errors/interchanges made in following maintenance years. The classical differentiation of cultivars based on morphological characteristics is a highly skilled and time-consuming task.

To assist in granting Plant Breeders' Rights for new potato varieties, a standard fingerprinting method has been developed (Reid & Kerr, 2007; Reid et al. 2009). It is a rapid and robust method for variety differentiation using nine microsatellite (SSR) markers. Over 1,000 cultivars have successfully been differentiated so far. Obviously, somaclonal variants and mutants cannot be separated from the original cultivar. The set of markers was expanded to twelve to give an added level of discrimination. All potato varieties maintained by SASA have been fingerprinted. SASA's potato SSR profile database is currently not public. This well established method will be applied for this AEGIS project.

The ECPGR European Potato Cultivar Database [EPCD] (www.europotato.org) currently lists information on 5,264 (presumable) different clones (incl. 4,000 cultivars as well as 159,000 observations) provided by 51 contributors. Those variety names that were used more than once for different genotypes (e.g. Gloria), are listed with year of release. When the identity is unclear then the abbreviation of the data donor is included in the name label.

The Multi-Crop Passport Descriptor list (MCPD) as well as EURISCO do not include a field for year of first release of varieties. In 2010 GRIN adapted the format of the downloadable passport data to provide this information. Obviously it is included in the EPCD.

4. Main objective and specific objectives

Establishment of the AEGIS collection for a part of the potato cultivars by means of fingerprinting old potato clones with questionable identity, to confirm or correct cultivar names.

5. Materials and methods

Material: 500 clones from different European collections (\neq SASA). Background knowledge: the SASA potato SSR database containing profiles of >1000 cultivars. Method: fingerprinting by means of 12 microsatellite (SSR) markers used in four multiplex reactions. Twelve markers (for 500 clones) will be used, giving the best discrimination. The use of the bare minimum of 9 markers (for 600 clones) will not be considered now additional funding for this fingerprinting work has become available from ECPGR, after a reallocation of funds from the Potato WG. This allows to increase the number of fingerprinted clones from 200 to 500.

Commercial labs offered to test a higher number of samples, but then a harmonization exercise would have been necessary, to allow the results to be compatible with those in the SASA database of potato SSR profiles, which can be a fairly drawn out and expensive process.

6. Expected outputs

The initial product of this project will be fingerprints of old potato clones from different European potato collections. Comparison with the SASA's extensive database on potato cultivar SSR profiles will identify mislabelling or confirm the genetic uniqueness of the clone, when no match with the database was found.

7. Benefits and impact

These results will significantly benefit the selection of potato MAA's for AEGIS. Curators will get essential information about the identity (and uniqueness) of the investigated germplasm. For the fingerprinted varieties a European Potato Collection will be proposed for AEGIS, indicating which accessions would be the Most Appropriate for inclusion.

8. Innovation

New are the fingerprints of previously not investigated genotypes (old varieties), which will be added to the database. Presumable parentage and offspring can be checked, when available in the database. The resulting SSR profiles from this project will be made public.

9. Application of results

Using the fingerprints, identities of old cultivars will be checked. The current identity will be re-identified, confirmed or recognized as a unique genotype when no match was found. Furthermore, new synonyms (or mutants) may be discovered, genetic distances can be calculated and presumable parentage and offspring may be checked, when available in the database. Last but not least: the selection of MAA's for AEGIS will be supported significantly and for the fingerprinted varieties a European Potato Collection will be proposed, indicating which accessions would be the Most Appropriate for inclusion.

10. Workplan

	Month 1-2	Month 3-6	Month 7-8	Month 9-10	Month 11-12
CGN (RH) and SASA (HC) selecting clones based on the ECPGR potato database and indications from curators	X				
Several curators (depending on the selection made in month 1-2) picking and drying leaves and sending samples to SASA		X			
SASA fingerprinting 500 clones			X		
SASA (AR) analysis and communication of results				X	
CGN (RH) establishment of Eur. Potato Collection for AEGIS for the fingerprinted varieties					X

11. Budget

The requested budget will primarily be used for DNA extraction and the fingerprinting work.

	project	in kind	Total
CGN staff time	1,000	1,000	2,000
potato curators staff time		1,000	1,000
curators - supplies	800		800
SASA staff time	16,000	1,000	17,000
“ lab supplies	9,000		9,000
Total	26,800	3,000	29,800

12. Contributions offered by applicant

- CGN (R. Hoekstra) and SASA (H. Campbell) will check the ECPGR potato cultivar database (www.europotato.org), request lists of questionable cultivar identities from curators and select 500 accessions (clones) from different European potato collection holders [non from SASA, because its collection is already fully fingerprinted, nor CGN (maintains no cultivars)].
- Curators from different European potato collections (e.g. IPK, INRA and others) will communicate the questionable cultivar identities within their collection, collect leaves from the ultimately selected clones, dry them on silica gel and send the dried leaf samples to SASA.
- Beside DNA extraction from the leaf samples and performing the routine fingerprinting using 12 SSR markers (100% project funded), A. Reid (SASA) will compare the fingerprinting results with the extensive SSR profile database and draw conclusions on the identity of the germplasm. SASA & CGN will inform the donors of the samples as well as the ECPGR potato cultivar database manager and make the resulting SSR profiles public.
- CGN (R. Hoekstra) will identify the proposed European Potato Collection for AEGIS concerning the fingerprinted varieties.

13. Bibliography

- Frese, L. & R. Hoekstra (2009). Report on a Network Coordinating Group on Sugar, Starch and Fibre Crops. Third Meeting, 8-9 October 2009, Quedlinburg, Germany
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www.plantbreeding.wur.nl/potatopedigree/
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- Reid, A., L. Hof, D. Esselink & B. Vosman (2009). Potato cultivar genome analysis. In: R. Burns (ed.), *Methods in Molecular Biology, Plant Pathology*, vol. 508, Chapter 23. p. 295-308. www.springerprotocols.com/Full/doi/10.1007/978-1-59745-062-1_23?encCode=U0xQQjMyXzEtMjYwLTU