



## **Collection and maintenance of elite rhizobial strains**

Milestone reference number 3.2.1

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## **N2Africa**

**Putting nitrogen fixation to work  
for smallholder farmers in Africa**



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## Introduction

One of the objectives of the N2Africa project is to improve the productivity of smallholder farms in sub-Saharan Africa through enhanced input of biologically fixed nitrogen in the farming systems. Biological nitrogen fixation (BNF) can be enhanced through a number of ways, including the use of highly effective rhizobial strains to inoculate legume crops. In this project, rhizobia for soybean (*Glycine max*), bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogaea*) will be isolated from indigenous populations in soils across the 8 countries where the project is operating and, using a stage-wise procedure, select strains with superior symbiotic and saprophytic competence as candidate inoculant strains. The isolation and selection of rhizobia strains, and their mobilization for inoculant delivery activities in the project, fall under Objective 3 as follows:

### **Objective 3: Select superior rhizobia strains for enhanced BNF and develop inoculum production capacity in sub-Saharan Africa through collaboration with private sector partners**

The component activities under this thematic objective are:

- 3.1. Assess the need-to-inoculate for the target legumes and identify elite strains across the impact zones.
- 3.2. Establish and characterize a rhizobium germplasm bank in the impact zones.
- 3.3. Formulate improved inoculant products and develop cost-effective production and delivery methods, including standardized quality assurance procedures.
- 3.4. Expand and upgrade inoculant production capacity in sub-Saharan Africa.
- 3.5. Conduct and advocate policy review on inoculant quality and cross-border movement.

Elite strains are determined by their superior performance to currently used inoculant strains, such that they are worthy of replacing those already in use as commercial legume inoculants. The major requirement for the choice of a strain for inoculant production is highly effective nitrogen-fixation with the intended host species under greenhouse and field conditions. Other beneficial characteristics include stress tolerance, competitive ability against the indigenous strains, genetic stability and satisfactory growth and survival during procedures for manufacture of inoculum (Howieson et al., 2000). In selecting for elite strains from local gene pools, it is important that the strains be identified and not confused with another and those already in use. Secondly, a comprehensive field testing is required to ensure that the candidate elite strains perform across a wide range of field conditions, with direct comparison to commercially-available inoculants. Finally, the compatibility of the strains within different inoculant production systems must be determined. Strains that are unique, consistently outperform currently available ones in the field and are readily incorporated into established production systems are eligible for release as elite strains.

In the entire laboratory to field continuum, all of the steps involved in the selection of elite strains require that the isolates undergoing evaluation are compared with those of the strains that are already in use as commercial inoculants or are considered as reference strains. This report documents the attainment of Milestone Activity 3.2.1 (**Elite strains obtained from leading inoculant producers and rhizobiology laboratories worldwide**), which requires the collection of elite rhizobial strains from various sources for use in cooperator laboratories to compare performance of isolates being selected as candidate elite strains.

## Background

Historically, scientists from international research centres, universities and leading inoculant manufacturers have routinely exchanged cultures of rhizobial strains for research and development activities. In this way, thousands of cultures are held in various collection



centres around the world, with CGIAR centres accounting for over 6000 of such collections (Street, 2000; Howieson, 2007). Exchange of cultures had prior to 1994 been undertaken without the written consent of legally constituted authorities in the country of origin since relevant international standards and national laws generally did not exist. There is now a general requirement for the preparation and signing of memoranda that deal exclusively with the acquisition, exchange, research and future commercialization of any strain (Howieson, 2007). In the case of the distribution of cultures from germplasm resource centres, requests for cultures may now often be met with Material Transfer Agreements (MTAs) that specify, amongst other things, that negotiation is required with the 'owners' of the material before commercial activities are to be undertaken. Usually, strains cannot be forwarded to a third party. An example of a current MTA is presented in Appendix 1.

Import regulatory and quarantine concerns are restricting the extent of germplasm exchange. Given their biological nature, trans-national movement of rhizobial products is generally subjected to a wide range of import regulatory laws. Although import of the rhizobial strains into countries is supposedly guided by a system that requires the receiver obtaining an import permit and the sender getting a phytosanitary certificate, getting the strains into the receiving countries are often beset with other policy constraints, which often are based on other considerations that may not be clearly spelt out by government officials in charge of regulatory agencies.

## Strain collections at cooperator laboratories

The 3 cooperator laboratories which serve as Rhizobiology hubs for N2Africa have varied collections of rhizobia strains, which constitute the initial sources of elite strains being used by the project. The Nairobi MIRCEN (Microbiological Resources Centre) at the University of Nairobi, Kenya, serves as the hub for East and Central Africa (ECA) region. The Centre has a collection of more than 250 rhizobial strains from local and foreign sources. Four initial strains are available from MIRCEN; these are *Rhizobium tropici* strain CIAT 899 (USDA 9030) and USDA 2667 for bean (*Phaseolus vulgaris*), and *Bradyrhizobium japonicum* USDA 110 and *B. elkanii* SEMIA 19 (CNPSo 9, BR29) for soybean (*Glycine max*).

The cooperator laboratory at the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria currently serves the West African hub and has a working collection of rhizobia strains out of which ten have been mobilized for N2Africa activities. These are *B. japonicum* 532c, USDA 4675, MAR 1496, and *Bradyrhizobium* spp. IRJ 2180A, RAUG 1, RACA 6, S(1), TSBF 560, TSBF 442 and TSBF 531. All of these are strains for soybean.

The Soil Productivity Research Laboratory (SPRL) Marondera, Zimbabwe serves as the hub for the southern Africa hub. The collection has over 500 rhizobial and bradyrhizobial strains of tropical and temperate origin among which three that are being used for inoculant preparation constitute the initial strains for N2Africa activities. These are *B. japonicum* MAR 1491 (USDA 110), MAR 1495 (USDA 122) and MAR 1496 for soybean

## Strain collections from other sources

EMBRAPA Brazil has approved Dr Mariangela Hungria's application for release of 4 elite strains for soybean to Mazvita Murwira (SPRL, Zimbabwe), Nancy Karanja (MIRCEN, Kenya) and Robert Abaidoo (IITA, Ibadan). The strains are to be released to these individuals and their organizations based on a material transfer agreement that is duly signed by them and the heads of their organizations (See Appendix 1). The strains are *B. japonicum* SEMIA 5080 (CNPSo 6 or CPAC 7) *B. japonicum* SEMIA 5079 (CNPSo 7 or CPAC 15), *B. elkanii* SEMIA 5019 (CNPSo 9, BR 29) and *B. elkanii* SEMIA 587 (CNPSo 14, BR96). SPRL Zimbabwe have received their consignment of the strains while those for MIRCEN Nairobi and IITA are still pending.



Eight strains were obtained from the culture collection of the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) Beltsville, Maryland and are being maintained at IITA. *B. (Arachis) spp.* USDA 3384, NC 92 and USDA 3451 (CB756) are for groundnut and cowpea; *R. tropici* CIAT 899 (USDA 9030) and *R. etli* USDA 9032 (CFN42) are for beans; and *B. japonicum* strains USDA 110, USDA 136 (CB 1809) and USDA 138 are for soybean. We are also currently processing an import permit for the transfer of eight strains from USDA-ARS collection to replenish the mother cultures at MIRCEN Nairobi for the MEA inoculant production facility at Nakuru, Kenya. The strains are USDA 2667, USDA 2669, and CIAT 899 for beans and USDA 110, SEMIA 5019, USDA 136 for soybean.

Efforts were also made to obtain 4 strains for cowpea, groundnut, bean and soybean from the Agricultural Research Council (ARC) Rhizobium Collection in South Africa but delivery has so far been delayed due to problems of paperwork. This is an example of the hitches being encountered at transferring strains across borders due to regulatory restrictions that impose the same conditions on products meant for research and those for commercial purposes.

## References

Howieson, J.G. 2007. Technical issues relating to agricultural microbial genetic resources (AMIGRS) including their characteristics, utilization, preservation and distribution. Working paper prepared for the genetic resources policy committee (GRPC) of the CGIAR. Food and Agricultural Organisation (FAO), Rome, 42 pp.

Howieson, J.G., Malden, J., Yates, R.J. and O'Hara, G.W. 2000. Techniques for the selection and development of elite inoculant strains of *Rhizobium leguminosarum* in southern Australia. *Symbiosis*, 28: 33–48.

Street, K.A. 2000. A discussion paper on the status of microbial genetic resources held by the CGIAR Centers. Unpublished internal review compiled by Dr Kenneth A. Street, ICARDA, on behalf of the CGIAR System-wide Genetic Resources Programme (SGRP), 25 pp.



## Appendix 1

### MATERIAL TRANSFER AGREEMENT – MTA

#### to be used when shipping genetic heritage samples for non-commercial research purposes

The Material Transfer Agreement (MTA) was established to monitor shipments of genetic heritage existing under *in situ* conditions, within the national territory, on the continental shelf and in the exclusive economic zone, or maintained under *ex situ* conditions, intended for Brazilian or foreign research institutions based on the following principles:

- Acknowledgment that the exchange of genetic heritage between research institutions in the field of biology and related areas, based in Brazil or abroad, is of vital importance to increase knowledge of Brazilian biodiversity;
- The need to ensure compliance with the provisions of the Convention on Biological Diversity, especially national sovereignty over biodiversity, prior informed consent and sharing of benefits arising from the use of genetic heritage.

Sending Institution: Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA
Address: Parque Estação Biológica – PqEB s/n°, Edifício Sede, Plano Piloto
Information on the representative of the Institution
Name: Pedro Antonio Arraes Pereira
ID (type, number, and issuing agency): ): CI n.º 2804840, SSP/RJ
Position of legal representative of the Sending Institution: President-Director
Legal document assigning authority to the legal representative: Nomination Decree

Receiving Institution:
Address:
Information on the legal representative of the Institution
Name:
ID (type, number, and issuing agency):
Position of legal representative of the Receiving Institution:
Legal document assigning authority to the legal representative: (attach a copy)

Project/Agreement in question (as appropriate): "Innovative strategies towards the increase of efficiency in the process of biological nitrogen fixation with grain and oil-producing legumes" (authorized by IBAMA in the special portfolio of Embrapa)

The signatory institutions, through their duly established representatives, bearing in mind the provisions of the Convention on Biological Diversity, Provisional Act No. 2,186-16, dated August 23, 2001, Decree No. 3,945, of September 28, 2001, as amended by Decree No. 4,946 of December 31, 2003, and Genetic Heritage Management Council Resolution No. 20, of June 29, 2006, undertake to use the sample(s) of the genetic heritage components transferred among themselves pursuant to the following conditions:



1. The material mentioned in Annex I of this Term must only be used by the receiving institution for non-commercial scientific research purposes, especially to studies of phylogeny, taxonomy and evaluation of nitrogen fixation and plant-growth promotion capacity of bacteria.
2. In cases of any subsequent wish to make use of the samples of the genetic heritage components transferred under this MTA for the purposes of bioprospection, technological development, or the request of a patent, the Receiving Institution shall undertake to so inform the Sending Institution, which shall in turn inform the Genetic Heritage Management Council or an institution accredited under the terms of Article 11(IV)(e) of Provisional Act No. 2,186, dated August 23, 2001.
3. Undertaking the activities mentioned in the previous paragraph without complying with the relevant legal provisions, and in particular without prior authorization from the Genetic Heritage Management Council, is prohibited.
4. Samples of genetic heritage components may not be transferred to third parties by the Receiving Institution unless a new MTA has first been signed between the original Sending Institution and the new Receiving Institution, in accordance with the provisions of Resolution no. 20, 2006.
5. Receiving Institutions shall abide by the terms of the MTA and shall not be considered providers with respect to the material received.
6. Any publication resulting from the use or study of shipped samples of genetic heritage components shall expressly acknowledge the origin of the material and credit the Sending Institution, to whom a copy of the publication in question must also be sent.
7. The Receiving Institution will facilitate access and transfer of technology to the Sending Institution or to another institution indicated by this, as a means of promoting the conservation and sustainable use of the genetic heritage transferred.
8. The Sending Institution is wholly responsible for identifying and properly packing the material, and for complying with specific shipment procedures related to biological risk assessment and for the containment of the organism or material transferred, observing all relevant official recommendations, international standards and specific legislation of the Receiving Country.
9. The Receiving Institution commits itself to:
  - a) not claiming any intellectual property rights over the genetic heritage components or parts of them transferred under the MTA, without prior access authorization issued by the Genetic Heritage Management Council;
  - b) b) informing the Sending Institution, in writing, of any adverse effects observed when handling the genetic heritage components under the MTA.
10. Failure to comply with the procedures set forth in this Agreement shall subject offenders to the penalties established in existing legislation.
11. The competent forum for settling disputes among institutions with respect to this MTA shall be the head office of the original Sending Institution.
12. The commitments related to the material transferred under this Agreement shall remain valid for an indefinite period of time, regardless of whether or not the Agreement has been renewed.



Having agreed with all the above provisions, the representatives of the Receiving Institution and of the Sending Institution hereby sign this Agreement, in three identical copies, each equally authentic, with equal legal effect.

Place and date: \_\_\_\_\_

<b>Receiving Institution</b>		<b>Sending Institution</b> Pedro Antonio Arraes Pereira – Director-President p/ Alexandre J. Cattelans

<b>Curator at the Receiving Institution</b>		<b>Curator at the Sending Institution</b> Mariangela Hungria



## Annex I – Qualitative and quantitative identification of the samples

Species	Strain #	Other designations	Type of material	#
<i>Bradyrhizobium japonicum</i>	CNPSo 6	CPAC 7, SEMIA 5080	lyophilized ampoule	02
<i>Bradyrhizobium japonicum</i>	CNPSo 7	CPAC 15 SEMIA 5079	lyophilized ampoules	02
<i>Bradyrhizobium elkanii</i>	CNPSo 9	29W, SEMIA 5019, 29w, BR 29	lyophilized ampoules	02
<i>Bradyrhizobium elkanii</i>	CNPSo 14	SEMIA 587, BR 96	lyophilized ampoules	02



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## List of project reports

1. N2Africa Steering Committee Terms of Reference
2. Policy on advanced training grants
3. Rhizobia Strain Isolation and Characterisation Protocol
4. Detailed country-by-country access plan for P and other agro-minerals
5. Workshop Report: Training of Master Trainers on Legume and Inoculant Technologies (Kisumu Hotel, Kisumu, Kenya-24-28 May 2010)
6. Plans for interaction with the Tropical Legumes II project (TLII) and for seed increase on a country-by-country basis
7. Implementation Plan for collaboration between N2Africa and the Soil Health and Market Access Programs of the Alliance for a Green Revolution in Africa (AGRA) plan
8. General approaches and country specific dissemination plans
9. Selected soybeans, common beans, cowpeas and groundnuts varieties with proven high BNF potential and sufficient seed availability in target impact zones of N2Africa Project
10. Project launch and workshop report
11. Advancing technical skills in rhizobiology: training report
12. Characterisation of the impact zones and mandate areas in the N2Africa project
13. Production and use of Rhizobial inoculants in Africa
18. Adaptive research in N2Africa impact zones: Principles, guidelines and implemented research campaigns
19. Quality assurance (QA) protocols based on African capacities and international existing standards developed
20. Collection and maintenance of elite rhizobial strains



## Partners involved in the N2Africa project



Caritas Rwanda



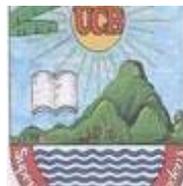
Diobass



Eglise Presbiterienne Rwanda



Resource Projects-Kenya



Université Catholique de Bukavu



University of Zimbabwe

