The International Treaty
ON PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE

# Full Project Proposal

Third Call for Proposals under the Benefit-sharing Fund

**MARKER ASSISTED SELECTION OF USEFUL CASSAVA GERMPLASM ADAPTED TO BIOTIC AND ABIOTIC STRESSES CAUSED BY GLOBAL CLIMATE CHANGE**

**(BIOCAS)**

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## PROJECT PROPOSAL COVER SHEET

Project No. _____ (*For Treaty use. Do not write anything here*)

Project Title: **MARKER ASSISTED SELECTION OF USEFUL CASSAVA GERMPLASM ADAPTED TO BIOTIC AND ABIOTIC STRESSES CAUSED BY GLOBAL CLIMATE CHANGE**

Project duration: **36 months**

Target crops: **Cassava (*Manihot esculenta*)**

Targeted developing country/ies        TANZANIA, KENYA

Other Contracting Party/ies involved     SPAIN

Project geographic extension (km²)     1.545.000

Total requested funding        **472.800 US$**

Total co-funding        **394.500 US$** (*In kind Contributions*)

**Please select the type of project you are applying for:**

☐ Single-country Immediate Action Project (Window 2)

☐ Multi-country Immediate Action Programme (Window 2)

☐ Single-country Co-development and Transfer of Technology project (Window 3)

☒ Multi-country Co-development and Transfer of Technology project (Window 3)

**Applicant**

Name of Organization: **Mikocheni Agricultural Research Institute (MARI)**

Type of organization        **Public Research Institute**

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# SECTION A: EXECUTIVE SUMMARY

## 1.        Executive summary

The project belongs to Window 3: Support to the co-development and Technology Transfer involving a Consortium of 3 partners from 2 East African countries and Spain. It addresses Cassava which plays a key role for food security and subsistence of farmers in many countries of Africa. Abiotic stresses and related biotic stresses caused by climate change represent a critical limitation and a major threat to sustainable agriculture and food security. It is necessary to develop new cultivars with tolerance to these stresses by exploiting the existing biodiversity of species. In this project we will characterize in part novel, yet unexploited Cassava germplasm from East Africa and identify accessions which are adapted to these threats of climate change. Based on this information we will develop molecular markers which can be used for Cassava breeding of new improved Cassava cultivars with elevated stress tolerance levels for sustainable agriculture.

Phenotypic evaluations of these accessions for resistance or tolerance to abiotic and associated biotic stresses will be performed in field trials and bio-assays. The traits for evaluation include abiotic stresses *cold, drought and heat as well as* the major viral diseases in cassava. The identification of tolerant genotypes will provide directly recommendations to farmers for cultivation of these varieties in environments with adverse agro-climatical conditions, and represent at the same time valuable material for the breeding of improved Cassava varieties.

On the other hand we will detect candidate genes (CG) for resistance or tolerance to these stresses using different up to date molecular tools. These include RNAseq, an in silico mining approach of known genes and RAD sequencing. We will analyse the allelic variation of these CG and determine the effect of specific alleles or allele combinations in the materials through amplicon sequencing and association mapping by linking the phenotypic data of the previous evaluations with the obtained molecular data. CG detection and analyses of alleles will be also performed using a random approach, known as RAD sequencing. The results will allow us to develop markers for marker assisted selection, which can be applied to speed up conventional Cassava breeding programs. Results of individual CG will be extended to multiple CG and combined for multiple traits through Model building with the practical aim of assigning parental breeding values and predict progeny performances in order to realize optimized crosses.

Pre-breeding activities by means of crossings and evaluations of resulting progenies will be performed to combine favourable characteristics and to improve adaptation to climate change, supported by the developed markers and models.

All Project results and Products (breeding clones) will be disseminated and transferred between partners, but also to farmer associations, to the scientific community, to breeders and to gene bank curators through numerous dissemination and transfer actions. A Project WEB page with an integrated Knowledge base will be established containing all project results and external links.

**Most important, extended training stages for technology transfer of trainees from the developing countries at the Lab in Spain, will allow these institutions to apply further or analogous studies independently on their own in the future.**

The molecular markers and Models for analysing stress adaptation in Cassava can be used for efficient marker assisted breeding in Cassava and related species.

## SECTION B: PROJECT DESCRIPTION AND CONTENTS

### 2.1. Problem definition

**Cassava (Manihot esculenta),** also called **yuca** or **manioc**, is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root, a major source of carbohydrates. Cassava is the third largest source of food carbohydrates in the world. Cassava plays a particularly important role in agriculture in developing countries—especially in sub-Saharan Africa—because it does well on poor soils and with low rainfall, and because it is a perennial that can be harvested as required. Its wide harvesting window allows it to act as a famine reserve and is invaluable in managing labor schedules. It also offers flexibility to resource-poor farmers because it serves as either a subsistence or a cash crop (FAO 2008).

The effects of global climate change such as heat, coldness, drought or flooding are threatening sustainable cassava cultivation. Moreover, changes in their affecting pathogen spectra have been observed. A number of serious diseases, such as cassava bacterial blight or cassava mosaic virus, affects cassava cultivation. Recently a new virus causing brown streak disease has been identified as a major threat to cassava cultivation worldwide.

Therefore, it is necessary to develop new cultivars that are adapted to these threats by applying marker assisted selection (MAS) or genetic transformations based on useful candidate genes.

The aim of this project is to characterize this valuable Cassava germplasm with respect to resistance and tolerance to different biotic and abiotic stresses and exploit it through breeding to obtain new Cassava varieties adapted to climate change for sustainable agriculture.

Genomic studies offer the possibility to characterize germplasm efficiently at the molecular level and to accelerate considerably breeding programmes. The detection of candidate genes for useful traits offers the possibility to apply them in marker assisted selection (MAS) within breeding programmes. The survey of allelic diversity of such genes within cultivated and wild accessions of a species and analyses of their particular effects, permits the selection of the most efficient allele combinations. Within this project, we want to identify in cassava useful candidate genes for different biotic and abiotic stresses using various molecular tools, characterize the allelic variation of this germplasm and use markers and models in marker assisted breeding in order to speed up the obtainment of improved varieties.

### 2.2. Project objectives: Overall and specific objectives

The **General Objective** consists of identifying Cassava accessions adapted to biotic and abiotic threats of climate change, and to identify the underlying candidate genes for developing molecular markers and models, which will speed up the breeding of improved and adapted Cassava cultivars for sustainable agriculture.

In order to meet this general objective the following **Specific Objectives** are envisaged:

1. Evaluation of Cassava accessions (cultivars, accessions, breeding clones) for resistance or tolerance to abiotic and biotic stresses related to global climate change.

2. Detection of useful candidate genes (CG) for abiotic and associated biotic stresses applying different molecular tools.

3. Molecular characterization of the allelic variation in these CG and determination of allelic composition in the evaluated accessions.

4. Association mapping to detect the effects of specific CG alleles or CG allele combinations on the tolerance levels of the analysed stresses, development of molecular markers for Marker-assisted selection and Model building to assign parental breeding values and predict progeny performances.

5. Pre-breeding activities in Cassava to combine favourable characteristics and to improve adaptation to climate change applying the developed markers and models.

6. Dissemination and Transfer of Project results and Products (accessions and breeding clones).

**2.2. Targeted outputs, activities and related methodology of implementation**

**Participants:**

**Three public institutions will carry out the R&D activities jointly:**

**P1. MARI** – Mikocheni Agricultural Research Institute (Lead Institution, Tanzania)

**P2. JKUAT** - Jomo Kenyatta University of Agriculture and Technology (Kenya)

**P3: NEIKER** - Basque Institute for Research and Development in Agriculture, Spain

**The following activities are foreseen, which are structured in 5 complementary work packages:**

| WP1: Phenotypic evaluation of the Cassava germplasm working collection |
| --- |

| Work package: | WP1 | Start: Month 1 | End: Month 24 | Duration: 24 months |
| --- | --- | --- | --- | --- |
| **Participants:** | P1: MARI | P2: JKUAT | | |
| **Man-months:** | 18 MM | 18 MM | | |
| **Milestones /Deliverables:** | M1.1, M1.2   /  D1.1ab,  D1.2ab | | | |

**Milestones / Objectives:**
**M1.1:** To carry out field trials to evaluate agronomic performance, resistance or tolerance to abiotic stress factors: drought, cold, heat, and to identify promising, adapted accessions.
**M1.2:** To carry out field trials and bioassays to evaluate resistance or tolerance to cassava mosaic, cassava brown streak viruses and Cassava bacterial blight and to identify resistant  accessions

**Description of work:**
**Task 1.1: Evaluation of resistance or tolerance to abiotic stress factors: drought, cold, heat**
Partners **P1, P2** will perform field trials as specified in **Table 1** below at locations with different stress conditions and at locations without stress (control), as well as in bio-assays under controlled conditions, using standard methodology.  A block design of single plant plots will be implemented, with four repetitions. The partners will record general agronomic performance, yield and starch content (or specific gravity) under normal and stressed conditions. In order to calculate stress tolerance levels, absolute and relative stress-induced yield losses will be computed. For combining the data from different trials, all values will be expressed as relative values with respect to the trial mean (100%).

**Task 1.2: Evaluation of resistance or tolerance to cassava mosaic, cassava brown streak viruses and Cassava bacterial blight**
Partners **P1, P2** will evaluate also the incidence of *Cassava* viruses and bacteria in the materials of the field trials at locations with high infection pressure. In addition, Virus and bacterial resistance will be determined according to Michalska et al. (2011) in bio-assays using detached leaves to complete the evaluations.

*General Output 1: Cassava varieties and accessions including Native Cassava species with resistance or tolerance to abiotic and associated biotic stresses related to global climate change identified, recommended for cultivation under adverse conditions and used for cultivation and breeding.*

| Task | Project outputs | Targeted Output (Deliverables) | Due date |
| --- | --- | --- | --- |
| *1.1.* | Results on evaluations of drought, cold and heat tolerance of the accessions through field trials and bio-assays. | **D1.1a,b:** Evaluation Data of at least 150 accessions & Recommended LIST of at least 20 accessions with tolerance to different abiotic stresses  for cultivation & breeding | Months* 12 and 24 |

| *1.2.* | Results of evaluation assays for resistances to viruses in the working collection. | **D1.2a,b:** Evaluation Data & Recommended LIST of at least 10 accessions with virus and bacterial resistance for cultivation & breeding | Months* 12 and 24 |
|---|---|---|---|

- *first results and final results, respectively*

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**WP2: Detection of useful candidate genes (CG) for abiotic and biotic stresses and Analysis of the allelic variation for these CG in Cassava**

| Work package: | **WP2** | **Start:** Month 1 | **End:** Month 24 | **Duration**: 24 Months | |
|---|---|---|---|---|---|
| **Participants:** | P1: MARI | P2: JKUAT | P3: NEIKER | | |
| **Man-months:** | 12 MM | 12 MM | 36 MM | | |
| **Milestones /Deliverables:** | M2.1, M2.2, M2.3, M2.4 / D2.1ab, D2.2ab, D2.3ab, D2.4ab | | | | |

**Milestones / Objectives:**

**M2.1:** To detect useful CG applying RNA-Seq in stressed and unstressed, susceptible and tolerant genotypes of Cassava

**M2.2:** To detect useful CG by analyzing published known genes from Cassava and other species.

**M2.3:** To perform successfully Amplicon sequencing of CG in a set of 150 accessions from the field trials (WP1) and to analyze the allelic variation in these Candidate genes.

**M2.4:** To perform successfully RAD sequencing in this set of 150 accessions, to extract additional CG with a relevant biological meaning and to analyze the allelic variation in the extracted CG.

**Description of work:**

Candidate genes will be detected initially using different molecular approaches and analysed for their allelic variability:

**Task 2.1 Library construction and RNA-Seq for CG detection**
Partner **P3** will perform this task, applying the following workflow: RNA will be extracted from selected susceptible and resistant genotypes cultivated under stressed (cold, drought, heat) and unstressed conditions. Barcoded strand-specific RNA-seq libraries will be constructed by partner **P3** according to Merrick et al. (2013) for each sample and multiplexed for sequencing using the Ion Torrent PGM platform. We will align RNA-seq reads using standard protocols to identify differences between treatments in sense and antisense transcript expression, splicing and allele-specific expression. Homology searches (via NCBI) will detect potential candidate genes with a relevant biological meaning.

**Task 2.2 Analysis of known candidate genes for biotic and abiotic stresses.**
Partner **P3** will perform in silico mining of sequence databases and publications in order to detect published candidate genes in Cassava but also in other species. In this latter case the Cassava homologs will be identified through BLAST searches against Cassava EST libraries.

**Task 2.3: Analyses of CG by Amplicon Sequencing (CG driven approach)**
Partners **P1**, **P2** will extract DNA from 70 accessions each, which are used for phenotyping in the field trials of WP1 and send them to Partner P3 (140 accessions in total).

Partner **P3** will design for each identified candidate gene from Tasks 2.1 and 2.2 appropriate primers in conserved exon regions based on the sequence information and homology searches and validate them initially in a small subset of genotypes by producing distinct and clear amplification products. Validated primers with a common extension will be used to produce amplicons in the set of 140 genotypes. The bands will be re-amplified via PCR in each genotype using specific barcode primers, which will allow to distinguish the origin (i.e: genotype) of each sample.

After verifying the quality of these final amplification products in gels, aliquots of each sample will be mixed in equal concentrations, and this mix of sample DNAs will be sent for sequencing using the "ION TORRENT Amplicon Sequencing" technique (Life Sciences).

After receiving millions of sequence reads from the Sequencing Platform (T2.3), Partner P3 will order and separate them by candidate gene and genotype. The number of different SNPs and patterns (alleles) which exist in the collection and their frequencies will be determined, as well as their frequencies in the population and the allele composition in each genotype of the collection.

**Task 2.4: RAD sequencing for CG detection and analyses (random approach).**
RAD (Restriction site associated DNA marker) sequencing and similar techniques (GBS, GWAS, Genomic Selection) allow potentially to identify many hundreds of CG at a time by screening the whole genome. We will apply a novel, modified RAD sequencing approach based on cDNA templates in order to capture the coding regions of the genome.

For this purpose Partners **P1**, **P2** will extract each also total RNA from the same 70 accessions of the field trials and send them to Partner P3.

**P3** will extract from each sample mRNA, perform reverse transcription and produce ds-cDNA samples. Restriction fragments will be produced by digesting with Ase + Taq. Following size selection and purification, appropriate adapters will be ligated. After re-amplification with barcoded fusion primers to identify later on the original genotype, the samples will be mixed in equal amounts and sent for Amplicon Sequencing using this time the novel ION PROTON technology (10 Gb chip).

The millions of obtained RAD sequences will be analyzed in a similar way as in T2.3. Restriction fragments will be extracted by homology searches and the allelic variability in terms of SNPs and Patterns (alleles) which exist in the collection will be determined for each extracted CG, as well as the allele composition in each genotype of the collection. Homology searches of RAD markers will be performed in order to identify potential CG with a relevant biological function for explaining stress tolerance.
Appropriate in-house developed Software is available for all analyses, but has to be adapted.

**\* In order to transfer the technology, a 6-months training stage of each African Partner is foreseen in this task.**

| | *General Output 2: Useful candidate genes for abiotic stress and associated biotic stress tolerance identified applying RNAseq, in silico Mining and RAD sequencing & existing allelic variation for these CG in the evaluated accessions determined.* | | |
|---|---|---|---|
| **Task** | **Description / Project outputs** | **Targeted Output (Deliverables)** | **Due date** |
| *2.1* | Results of CG analyses derived from RNAseq sequences for drought, cold and heat tolerance. | **D2.1a,b:**List of at least 100 new candidate genes for abiotic and related biotic stress resistance derived from RNAseq. | Months* 12 and 24 |
| *2.2.* | Results of *in silico* mining to detect published candidate genes for tolerances to abiotic and biotic stresses. Identification of homologues in Cassava. | **D2.2a,b:** List of at least 50 Cassava CG derived from in silico mining of published candidate genes for abiotic and related biotic stress resistance. | Months* 12 and 24 |
| *2.3.* | CG sequences and Amplicon primers. Results of allelic variation of CG and allele composition of the accessions derived from Amplicon sequencing. | **D2.3a,b:** List of CG Sequences and functional primers for obtaining CG amplicons. For each CG LIST of SNP/alleles in the collection and CG allele composition of each accession. | Months* 12 and 18 |
| *2.4.* | Results of CG extractions derived from RAD sequences, allelic variation of CG and allele composition of the accessions | **D2.4a,b:** LIST of CG extracted from RAD tags and their biological meaning. LIST of at least 300 SNP/alleles in the collection and CG allele composition of each accession. | Months* 18 and 24 |

| WP3: Association Mapping and Model Development |
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| Work package: | WP3 | Start: Month 20 | End: Month 36 | Duration: 16 months | |
|---|---|---|---|---|---|
| Participants: | P1: MARI | P2: JKUAT | P3: NEIKER | | |
| Man-months: | 9 MM | 9 MM | 24 MM | | |
| Milestones /Deliverables: | M3.1, M3.2, M3.3 , M3.4 / D3.1ab,  D3.2ab, D3.3ab, D3.4 | | | | |

**Milestones / Objectives:**
**M3.1:** To perform Association Mapping for detecting associations of specific marker alleles with levels of stress tolerance in the set of 140 accessions from the field trials (WP1).
**M3.2:** To design allele specific primers (ASP) for important CG alleles
**M3.3:** To develop models for marker assisted selection (MAS) by assigning parental breeding values and progeny performance predictions.
**M3.4:** To validate and refine these models based on observed progeny performances in Task 4.2

**Task 3.1- Association Mapping to detect CG allele effects**

Based on the results from Tasks 2.3 and 2.4, Partner **P3** will analyse the potential effects of specific candidate gene alleles (or combinations of such alleles) on the phenotypic expression of the corresponding trait. For these purposes association mapping techniques based on LDA ("linkage disequilibrium analysis"; Luo et al. 2000) will be applied according to Yu et al. 2006  and Abdurakhmonov & Abdukarimov 2008, and particularly using the mixed-model approach (Stich et al. 2008).

**Task 3.2 Design of allele specific primers (ASP) for important CG alleles**
Based on observed sequence differences between CG alleles it is possible to design primers, which amplify selectively only specific alleles. ASP development is complex and multiplexing is difficult due to varying, specific PCR conditions. Therefore, Multiplexed PCR and Amplicon sequencing will be the method of choice with dropping sequencing costs when analyzing a larger number of CGs.
Nevertheless, Allele-specific primers will be designed for single alleles of selected important genes, which contribute very significantly to phenotypic trait expression. These can be used for rapid screening of individual CG alleles. Their functionality will be evaluated in test amplifications on a small subset of the original genotype set.

**Task 3.3 Initial Model Development**
Based on the **results from Task 3.1**, we will perform Model building for MAS considering multiple CG, These Models will allow to estimate parental Breeding Values (**BV)** and to predict Progeny performance (**PPP**).

Novel technologies for model building using specialised bioinformatics Software will be used for this purpose.

We will establish **cualitative** and **quantitative Allele Models** (AL) and **Allele Combination Models** (AC) using multifactorial analyses (**Proc GLM, Proc Mixed**) applying the following steps.
1. **Selection of specific CG for the Model**, based on significances of individual CG effects and CG value correlations.
1**. Assignment of values to alleles and AC** based on the performance of the genotypes (GT) where they appear.
3. **Assignment of Parental Breeding values** (**BV**; only for AL Models) based on the average value of the alleles of each parent, averaged over all selected CGs)

We will establish **PREDICTIONS of PROGENY PERFORMANCE**:

For **AL Models** = based on the Average Parental Breeding Value
For **AC Models** = Average AC value of expected AC depending on parental alleles, averaged over all selected CG

The best Model is supposed to have the highest correlation between predicted and observed values. In-house developed Software (**TAMAS**) is available for these analyses and will be adapted.
Based on these predictions an initial List of recommended crosses will be established. This list will be used for performing the crosses in the 3ʳᵈ year (Task 4.2)

**Task 3.4 Model Validation and Refinement**

**A) Model Validation:** Using the parameter estimates of **T3.3** for each CG and the molecular data (allele composition of GT) from **T2.3 and T2.4** it is also possible to make Progeny Performance Predictions for the progenies from Task 4.2, below.

On the other hand NEW, observed progeny performance data for the traits of interest will be obtained in Task 4.2. Thus, by comparing predicted model data (PV, PPP) and observed phenotypic data of the Progenies from Task 4.2, it is possible to validate and proof the general applicability of a MAS Model, if significant correlations are obtained. Therefore, alternative MAS models will be validated based on correlation analyses, by paired T-Tests or Wilcoxon signed-rank tests using SAS Software (SAS 1989). The BEST MAS model can be determined in this way.

**B) Model Refinement:** The phenotypic data from Task 4.2 will allow establishing new models as described in Task 3.3, but here based on the expected allele configurations in the progeny genotypes, and allow to estimate new PV and PPP values. By combining the results of the initial validated models and these new models, it will be possible to refine the existing Models for optimal predictions.

**\* In order to transfer the technology a 6-months training stage of each African Partner is foreseen in this task.**

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|---|---|---|---|
| *General Output 3: Effects of specific CG alleles or CG allele combinations on the tolerance levels of the analysed stresses detected through Association Mapping and Models allowing predictions of parental breeding values and progeny performances established.* | | | |
| **Task** | **Project outputs** | **Targeted Output (Deliverables)** | **Due date** |
| *3.1. Analysis of potential associations of specific marker alleles with specific characteristics* | Results of potential associations of specific marker alleles or combinations with specific characteristics such as tolerance levels and production | **D3.1a,b:** LIST of at least 100 effects of alleles and AC of each CG on stress tolerance levels and production. | Months* 24 and 32 |
| *3.2 Allele specific primers* | Results of the PCR assays of allele specific primers for important CG alleles | **D3.2a,b:** LIST of at least 30 allele specific primer sequences and their applications | Months* 24 and 32 |
| *3.3. Initial Model Development* | Identification of powerful models to predict progeny performances with respect to tolerance to abiotic/biotic stresses and production. | **D3.3a,b:** Four novel technologies for model building using specialised bioinformatics Software developed. Initial ESTIMATES of Parental Breeding values and Progeny performances for different models. LIST of most promising recommended crosses for the 3ʳᵈ year. | Months* 24 and 32 |
| *3.4 Model Validation and Refinement* | Results of Model Validation and Model Refinement | **D3.4a,b:** Model PARAMETERS of optimized models. LIST of most promising crosses recommend for the future, based on these results. | Month 36 |

| **WP4: Pre-breeding activities to combine favourable characteristics, to improve adaptation to climate change, and to improve progeny performance predictions** |
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| Work package: | WP4 | **Start:** Month 12 | | **End:** Month 36 | |
| --- | --- | --- | --- | --- | --- |
| **Participants:** | P1: MARI | P2: JKUAT | | | |
| **Man-months:** | 9 MM | 9 MM | | | |
| **Milestones / Deliverables:** | M4.1, M4.2, M4.3   /  D4.1ab,  D4.2ab, D4.3a,b | | | | |

| **Milestones / Objectives:**<br>M4.1: To perform crosses between Cassava accessions from the field trials (WP1)<br>M4.2: To evaluate the resulting progenies in the field.<br>M4.3: To apply the developed allele-specific primers for progeny genotype selection. |
| --- |

**Description of Work:**

**Task4.1 Performance of crosses between accessions from WP1**

Partners **P1**, **P2** will perform each year crosses using the accessions which are evaluated in WP1 as parents. The aim is to combine favorable characteristics of the parents or even superior CG alleles with respect to stress tolerance. For the crosses of the 3$^{rd}$ year, the recommendations provided by the initial Models (Task 4.3) will be applied.

**Task 4.2 Evaluation of the obtained progenies for agronomic performance and tolerances**

The obtained progenies will be sawn in the field at locations with adverse conditions and evaluated for their agronomic performance in order to select genotypes with superior characteristics. Participatory selection involving local farmers will be applied.

**Task 4.3 Application of the developed allele-specific primers for genotype selection.**

At the same time the allele-specific primers which have been developed in Task 3.2 will be applied to check the most promising genotypes in the progenies for the presence of favourable alleles.

- *Evaluation data will be also used for model validation and refinement as described in detail in Task 3.3.*

| *General Output 4: Genotypes with combined favourable characteristics obtained through pre-breeding activities and application of developed markers, allowing to improve adaptation to climate change and progeny performance predictions.* | | | |
| --- | --- | --- | --- |
| **Activities** | **Project outputs** | **Targeted Output (Deliverables)** | **Due date** |
| *4.1. Crossings* | Crossings between promising accessions in order to combine favourable characteristics | **D4.1a,b,c:** List of performed crossings (at least 200)  and promising parents (at least 30) involved | Months* 12, 24 and 36 |
| *4.2. Evaluation of resulting progenies* | Results of progeny evaluation and Selection of superior breeding clones with combined favourable characteristics. | **D4.2a,b:** Data of Progeny evaluations, LIST of selected progeny genotypes. At least 60 breeding populations developed and 9 advanced breeding clones identified. | Months* 24 and  36 |
| *4.3. Application of MAS* | Results of application of the developed allele specific primers in selected progeny genotypes. | **D4.3a,b:** Data of at least 100 marker validations in selected genotypes | Months* 24 and  36 |

| **WP5: Dissemination and Transfer of Project results and Products** |
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| Work package: | WP5 | **Start:** Month 1 | | **End:** Month 36 | |
|---|---|---|---|---|---|
| **Participants:** | P1: MARI | P2: JKUAT | P3: NEIKER | | |
| **Man-months:** | 16 MM | 16 MM | 4 MM | | |
| **Milestones /Deliverables:** | M5.1, M5.2, M5.3, M5.4, M5.5/D5.1, D5.2, D5.3, D5.4, D5.5 | | | | |

| **Milestones / Objectives:** |
|---|
| **M5.1:** To establish a Knowledge Base on Tolerance/ Resistance to stresses in Cassava<br>**M5.2:** To establish  a Project WEB page<br>**M5.3:** To disseminate project results through publications and congress presentations<br>**M5.4:** To transfer project results and products between partners and to the Cassava productive chain<br>**M5.5:** To establish Demonstrative plots of most promising accessions or breeding clones. |

**Description of work**

The consortium members consider several instruments and numerous actions to efficiently disseminate, transfer and exploit technology, knowledge, materials and other project results.

**Task 5.1: Establishment of a Knowledge Base on Analysis and Evaluation of Resistance / Tolerance to stresses in Cassava**
All phenotypic and molecular data obtained in the project, the results of association mapping and model building including the applied methodology will be compiled into a Knowledge Database "Analysis and Evaluation of tolerance/ resistance to stresses in the Cassava crop." All partners will provide the necessary input.

**Task 5.2: Establishment of a Project WEB Page**
Participant **P3** will establish the Project WEB page: "BIOCAS" with information about the project and its partners, along with all results, which are being obtained. The Knowledge Base will be part of this website. For this purpose, all participants will send relevant information and results to P3.

**Task 5.3: Dissemination at the scientific / technical level**
Scientific and informative articles, contributions to conferences and training courses will be realized. For internal transfer between partners, the annual meetings will be combined with technology transfer courses. The technologies will include phenotypic and agronomic assessments, specific molecular techniques, bioinformatics and statistical methods.

**Task 5.4: Transfer between partners and to the sector (productive chain)**
**Each partner will send 1 appropriate trainee 2 times for 6 month stages for training ("learning by doing"). These trainees will have an important role to transfer and implement all technologies in their partner institutions, so that they qill be able to conduct the analyses by their own in the future!**
On the other hand Fairs and Field Days with farmers, breeders, experts and other actors of the Cassava productive chain (stakeholders) will be organized to inform about the project and project results, to show the field trials, to present adapted varieties and to distribute planting materials of recommended varieties for cultivation.
Regional workshops will be held with farmers and farmer associations to present and discuss new knowledge and practices. Recommendations for growing Cassavas and proper handling will be given.

**Task 5.5: Establishment of Demonstration Plots**
Demonstration fields will be established in harsh environments in order to show adapted varieties / breeding clones with a good agronomic performance under these conditions. Thus, farmers and experts can check the value of these varieties for sustainable agriculture.

| | | | |
|---|---|---|---|
| **General Output 5: Efficient Dissemination and Transfer actions realized to implement successfully Project results and Products.** | | | |
| **Task** | **Project outputs** | **Targeted Output (Deliverables) \*** | **Due date** |
| *5.1. Knowledge Base* | Establishment of a Knowledge Database about "Analysis and Evaluation of tolerance/ resistance to stresses in the Cassava crop. | **D5.1:** Knowledge Database based on all project results and external information | Month 6 and updates month 12, 24,36 |
| *5.2. Project WEB page* | Establishment of an informative Project web page | **D5.2:** Project WEB Page "BIOCAS" with regular updates | Month 6 and updates month 12, 24,36 |
| *5.3. Scientific Dissemination* | Efficient transfer of project results between project partners and to the scientific community | **D5.3:** Publications of project results. Presentation of project results in conferences and workshops (at least 20). | Periodically during the project |
| *5.4. Transfer of results to the sector* | Efficient transfer actions between partners and to the sector (productive cahin) and stakeholders | **D5.4a:** Extended Training stages of Partners from developing countries at NEIKER | 1 trainee from each partner 2 times for 6 months (Year 2 and 3) |
| | | **D5.4b:** Fairs, Field Days, Regional Workshops. Distribution of planting material | Periodically during the project |
| *5.5. Demonstration Plots* | To establish Demonstration plot s with most promising varieties or breeding clones | **D5.5:** Demonstration Plots of adapted varieties /breeding clones | Months 30 to 36 |

**\* In addition the Targets specified in the Log Frame (Appendix 2)**

### 2.4. Targeted PGRFA

The project targets the Cassava crop in a broad sense and will include commercial cultivars, local accessions or landraces and breeding clones.

The following plant material will be used in the project, or produced (= progenies) within the pre-breeding activities. In addition, the location of the field trials is indicated and their characteristics (**Table 1**).

| **Partner** | **Nº of Accessions\*** | **Field Trials for Evaluation** | | | **Pre-Breeding** |
|---|---|---|---|---|---|
| | | **Location** | **No of Acc.** | **Stress** | **Nº of crosses/ Progenies** |
| P1 MARI | 1.= 25 2. =50 3. =20 | Field at Chambezi/Field at Hombolo | 60 60 | Drought /Control Cold | 120/60 |
| P2 JKUAT | 1. =30 2. =30 3. =30 | Greenhouse at JKUAT Field at Kakamega Field at Mtwapa | 50 50 50 | Heat/Drought Control/Drought Cold | 100/50 |

- *1. Commercial Cultivars, 2. Accessions, 3. Breeding clones,*

### 2.5. Target groups and beneficiaries

The East African farmers will have **already after one year** of project execution recommended cultivars or accessions at their disposal that can be grown under harsh and adverse agro-climatical conditions. In the near future through the pre-breeding activities in this project, Cassava varieties with improved properties such as tolerances and resistances to abiotic and associated biotic stresses which are adapted to the global climate change are obtained for sustainable agriculture. Supported by the different planned dissemination actions we expect to target over 500 farmers and their families in each country.

The knowledge and molecular data generated by the project are useful to increase the information about the entries of a Germplasm Bank, and can be integrated in the passport data. They provide guidelines for the functional biodiversity conservation of useful gene alleles for characters of interest, improving the representativeness and usefulness of the entries in a Germplasm Bank. The available information increases the use of such banks by the breeders, due to the availability of markers for assisted selection in genetic improvement programs.

Cassava breeders will have improved breeding clones as progenitors at their disposal which can be used to develop novel Cassava varieties. We will address at least 20 Curators of Germplasm Banks and 30 Cassava breeders.

Breeders and researchers will have a set of molecular markers and predictive models useful for assessing adaptation to abiotic stresses in germplasm, progenitors and breeding clones at their disposal, which can be used to develop novel Cassava varieties.

The applied concept, using Cassava as a model species of the genus *Manihot* can be potentially applied to other related species and crops.
The project WEB page and the foreseen congress presentations of project results will allow targeting at least 500 scientists.


### 2.6. Impact and impact pathways

#### 2.6.1. Food security and poverty alleviation

Our project will identify in a short-term varieties and accessions with better adaptation to adverse environmental conditions which are suitable for cultivation in disadvantaged zones. Thus, our project will contribute to the adaptation of the Cassava crop to the possible threats posed by climate change and prevent significant losses in production. These threats are closely related to heat, cold and water availability and increased incidences of pests and diseases.

The cultivation of suitable genotypes will increase the income of farmers, thus contributing to sustainable development, food security and sovereignty and increase the quality of life and peace in this region.

The project will develop in a medium-term through the foreseen breeding activities also new Cassava cultivars with even higher tolerance levels to the analysed stresses, by combining favourable characteristics of the progenitors. Thus, at the end of the project farmers will have improved Cassava varieties adapted to extreme climatic conditions for sustainable agriculture at their disposal. This will lead to additional income, improved life standards and increased financial capacities for new investments or additional purchase of consumables.

The numerous foreseen dissemination and transfer actions to farmers and all actors of the Cassava chain will ensure the efficient implementation of all project results and products.

#### 2.6.2. Adaptation to climate change and environmental sustainability

The availability of suitable varieties for adverse environmental conditions will improve the competitiveness of the Cassava crop, increase the area of cultivation and diversify agricultural production.

Through the provision of varieties tolerant to extreme environments, the project aims to contribute to the Millennium Development Goals (1,7 and 8). It will enable to expand the agricultural frontiers for Cassava cultivation and will favour inhabitants in regions with extreme climates, allowing them access to new sources of nutrition and income. At the same time this will allow to cultivate areas that previously did not have alternative crops, reducing in this way the impact of desertification.

#### 2.6.3. Scientific impact

The knowledge and materials generated by this project will accelerate significantly Cassava breeding programmes to obtain improved varieties for sustainable agriculture adapted to climate change.

The specific candidate gene alleles with low and high values for adaptation to climate change which will be detected, provide guidelines for functional germplasm conservation to gene bank curators which should aim to particularly conserve the most useful CG alleles. Valuable accessions will be maintained in this way and can be exploited by Cassava breeders.

However, the predictive models developed in this project will have a very practical impact, far beyond the application of several individual markers for MAS, by suggesting exactly the most promising crosses which should be performed for most efficient breeding.

### 2.6.4. Capacity development and empowerment

The foreseen transfer of all generated project results, knowledge, methodologies and Software will strengthen the capacities of the partners in this project, which in turn will transfer the acquired items to their national researchers and project target groups.

After project completion they will be able to launch analogous projects in other related crops or other research topics using the applied strategy methodologies of this project.

## 2.7. Relevance to national or regional priorities in its plans and programmes for PGRFA

The governments of the East African countries have made tremendous efforts to protect and use biodiversity in a way that can ensure its use without adversely affecting the natural habitats. Bio-prospecting plays an important role in conservation and utilization of these resources. For Cassava, the characterization and safeguard of the local landraces growing in EAST Africa will allow its use for the Cassava farmers and will reduce the environmental impact of the excessive use of agrochemicals.

The governments of Tanzania and Kenya have launched National Food Plans, which give special importance to the cultivation of the Cassava. It is therefore strategic for the countries, to count on new varieties adapted to the increasing threats related to climate change and its ecological and economic implications. In Kenya particularly, efforts have been focused towards the development of high yielding varieties that are tolerant to pests and diseases, among other factors. At the same time, viable alternatives for the production and export of Cassava to other countries have emerged, either as seed or for fresh consumption. The participating institutions have taken a leading role in the new government strategies and therefore on the breeding and cultivation of Cassava. The Governments have therefore initiated appropriate policy measures that will make cassava a commercial crop so as to match the dynamic changes in the industry and the world at large. In Tanzania cassava improvement efforts involves breeding for high yielding as well as tolerant to pest and disease that threaten loss of cassava biodiversity. The focus has been to control cassava mosaic disease and cassava brown streak disease.

For NEIKER the interest lies in the knowledge about candidate genes for climate change and molecular markers which will be generated through this project. These can be transferred to or exploited in other genetic backgrounds and even related crops.

## SECTION C: OPERATIONS

### 3.1. Methodology of project implementation

Right at the beginning of the project a Consortium Agreement will be signed between partners in order to establish the legal and technical aspects of the collaboration.

We will apply up-to-date Methodology to implement the Project (see: The Basics of Project Implementation, CARE US;
http://www.careclimatechange.org/files/toolkit/CARE_Project_Implementation.pdf).
The components of a successful project include managing relationships with various stakeholders, managing human resources, managing financial resources, facilitating learning, managing risks and ensuring flexibility.

Some of the elements for project implementation are already included in this proposal, such as objectives (2.1) a detailed work plan (2.2) including activities and executing partners, expected outputs and time schedule of tasks, project budget (Appendix IV) and a logical framework (log frame) that explains how the project will contribute to an ultimate impact  (Appendix  III).
Others will be implemented at project start, such as a Monitoring and Evaluation Plan, Budget Planning and Monitoring including a Staffing & Procurement Plan. Moreover, an Annual Work Plan (AWP) will be prepared, containing a detailed planning of the foreseen activities and deliverables and the specific set of results to achieve during a particular year. In order to control and monitor the progress of the project the following elements will be implemented:

### Project Management Board  (PMB)

The PMB will monitor the progress of the project. It is composed of the Coordinator and the principal investigators of each participating institution, together with the corresponding heads of departments of each institution as external observers and project staff, if required.
The coordinator in collaboration with the other PMB members will be responsible for reporting and the technical and financial management of the project.

### Communication flow and Progress Control

General communication between the project partners will be realized via email or Skype. At each anticipated milestone the responsible partner(s) will write a brief report, stating whether the milestone has been met and, if not, the reason and a new expected date when the milestone will be met. In addition, to enhance the exchange of data and ideas and to enable the coordinator to closely follow the progress (and interfere if necessary) it is expected that each partner regularly provides the coordinator with informal progress reports, who will take care of the distribution of the information via email.
In order to facilitate the monitoring of activities and progress, a chronogram for the planned R&D tasks has been established (Appendix II).
Reporting in appropriate format will be performed as requested by the financing agency.
Annual meetings will be realised to plan and coordinate the R&D activities, to present and discuss the obtained results and to realize TT courses.

### Management of knowledge and intellectual property

The project generates new knowledge in different fields as described above. Several instruments are implemented to disseminate freely this knowledge and associated technologies and results. These include beside publications also congress contributions and a detailed Project WEB page. No restrictions exist upon the diffusion of results and germplasm.

### 3.2. Partnerships and collaboration arrangements

The Leader of this research project is **Mikocheni Agricultural Research Insitutute**  (Dar Es Salaam, Tanzania). MARI is at the forefront of scientific research in Tanzania and started its activities in 1998. One of the main research areas at MARI focuses on the economic potential of native species (including Cassava). MARI has previous experience in field research and laboratory research concerning the use of molecular techniques to identify and characterize germplasm resources of different species. MARI collaborates in this project with the co-participant **Jomo Kenyatta University of Agriculture and Technology** (JKUAT) - Department of Horticulture (Nairobi, Kenya ).

The main activities of JKUAT include agricultural R&D and the application of scientific knowledge and technological innovations focusing on the rational exploitation and preservation of natural resources including Cassava.

Both partners collaborate in this project with NEIKER (Basque Institute for Research and Development in Agriculture; Vitoria-Gasteiz, Spain) which provides the Technology Transfer. NEIKER has realized different genomic studies in a dozen of plant species applying different molecular tools such as transcriptome mapping, differential cDNA-AFLP, microarray analyses or EST sequencing from enriched libraries. Actually NEIKER participates in large Genomic Projects in oil palm and *Acacia*. Knowledge about markers, software and methodologies to perform the planned R&D activities of this project have been applied successfully in these crops.

**The collaboration with NEIKER is absolutely necessary, for transferring and implementing the Technologies and Results, which otherwise would not be possible. Considering the elevated labor costs /unit in Europe, the budget for these activities is very moderate. Consumable and Sequencing costs are necessary to achieve the results. The main benefits are off course for the Developing Countries.**


**3.3. Project management team**

The composition of the project management team is given below for each collaborating institution:

**P1. MARI (Tanzania):**

**Prof. Dr. JOSEPH NDUNGURU** is Principal Scientist at MARI as well as Adjunct Professor of Nelson Mandela African Institute of Science and Technology, Arusha, Tanzania. He has significant experience in the field of Plant Pathology, specifically the genetic and molecular basis of resistance to plant diseases, and the evaluation and sustainable use of genetic resources combining biodiversity and modern biotechnology. As project coordinator, he will be responsible for the overall technical and financial project management, the performance and evaluation of the field trials and the dissemination and promotional events.

**Dr. Gladness Elibariki** is a researcher at MARI with more than 15 years' experience particularly in genetic resource management, molecular biology and genetics. Her PhD research involved partly molecular genotyping of cassava landraces in Tanzania. She will be responsible for the breeding activities and molecular analysis.
**N.N. Master Students, Technicians and field workers** will collaborate in field trials and phenotypic evaluations.


**P2. JKUAT (Kenya):**
**Dr. Elijah Ateka,** An Associate Professor  has 17 years' experience in Plant Pathology (Virology) working on the biotic constraints to cassava production. He has participated in breeding for resistance and quality traits in Cassava. He is responsible for the Integrated Cassava Crop Technology in the Department. He will be responsible for the technical and financial project management at JKUAT and for dissemination actions and promotional events.

**Dr. Edward Mamati** has 13 years' experience in breeding for resistance to viruses and bacteria. He will establish the germplasm collections and perform filed trials and breeding activities.

**N.N. Technicians, Field workers** and **Master Students** will collaborate in all project tasks.


**P3: NEIKER**
**Dr. E. Ritter** has over 20 years' experience particularly in breeding, molecular biology and genetics, and has participated in and managed many international projects. He will be responsible for the technical and financial management, the statistical analyses and the dissemination and promotional events.
**Dr. JI Ruiz de G.**  is researcher at NEIKER and has over 15 years' experience in breeding of different crop species. He will be responsible for the bioassays and in silco mining techniques and collaborate in the statistical analyses.
**Dr. L. Barandalla** has 12 years' experience in molecular marker technology as part of many previous R&D projects. She will be responsible for the generation of molecular data (molecular analysis)

NN additional **researchers** of the scientific team and **technicians** with relevant experience will collaborate in specific project tasks.

### 3.4. Sustainability

All partners have the necessary land and lab facilities as well as adequate financial, human and institutional capacities to perform successful the planned R&D activities in a sustainable way. Moreover, they know each other well and have already collaborated in the frame of other international R&D projects. They are also well connected to the government and all actors of the Cassava chain, ensuring in this way an efficient implementation of the project results and products.

The project will allow the interaction of researchers from developing and developed countries, which will be of great benefit to both parties. This would settle the basis for future joint projects and the effective dissemination of results. It also gives an important added value to native Cassava accessions which are hitherto unknown, and that greatly enhance the development of new Cassava varieties with tolerance to the major abiotic stress factors. Additionally, this rresearch project will stimulate further collaborations between project partners on the same or related topics.

Candidate genes and markers developed in this project can be exploited in other related (wild) species and probably also in more distant species.

Availability of knowledge, materials and markers will improve considerably the competitiveness of the collaborating institutions as partners for such further R&D projects.

## SECTION D: APPENDIXES

By signing this submission form for full proposal, the applicant confirms that all the above statements, including the attached Appendixes, are true to the best of his/her knowledge. Any deliberately untruthful response will lead to the automatic exclusion from the further screening and appraisal process, and may lead to the denial of awarded grants from the Benefit-sharing Fund.
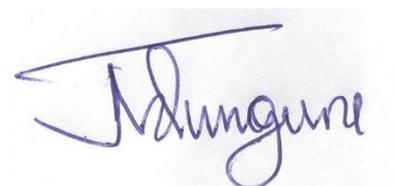
**28/11/2014**

**Signature of contact person:**                             **Date and location**