RPF - I

## (PROFORMA FOR SUBMISSION OF RESEARCH PROJECTS)

### Part - I: General Information

**200 Project Code:**
2001 Institute Code No. : CI 6.8
2002 ICAR Code No. :

**201 Name of Institute and Division:**

2011 Name & Address of Institute: **Indian Grassland and Fodder Research Institute**
**Gwalior road, Jhansi 284003 (UP)**

2012 Name of Division/Section: **Crop Improvement**

2013 Location of Project : Indian Grassland and Fodder Research Institute, Jhansi

**202 Project Title:**'' Development of molecular markers database in forage grasses with special reference to stress responsive EST-SSRs"

**203 Priority Area**: Basic research

2031 Research Approach: Applied Res. / **Basic Res.** / Process /TOT of Tech.Develop.
01 **02** 03 04

**204 Specific Area**: Plant Biotechnology

2041 Previous Project/Projects in this specific area: Nil
(Year, type of funding, cost, etc.)

**205 Duration**: 03 yrs

2051 Date of Start: 01-10-12

2052 Likely Date of Completion: 30-09-15

**206 Total Cost of the Project:**

2061 Foreign Exchange Component (If any):

## Part - II: Investigator Profile

**210 Principal Investigator:**

| | | |
|---|---|---|
| 2101 | Name | : A. Radhakrishna |
| 2102 | Designation | : Scientist (Biotechnology) |
| 2103 | Division/Section | : Crop Improvement |
| 2104 | Location | : Indian Grassland and Fodder Research Institute (IGFRI) |
| 2105 | Institute Address | : Indian Grassland and Fodder Research Institute<br>Gwalior road, Jhansi 284003 (UP) |

**211 Co-investigator:**

| | | |
|---|---|---|
| 2111 | Name | : K.K. Dwivedi |
| 2112 | Designation | : Senior Scientist (Biotechnology) |
| 2113 | Division/Section | : Crop Improvement |
| 2114 | Location | : Indian Grassland and Fodder Research Institute (IGFRI) |
| 2115 | Institute Address | : Indian Grassland and Fodder Research Institute (IGFRI)<br>Gwalior road, Jhansi 284003 (UP) |

**212 Co-investigator:**

| | | |
|---|---|---|
| 2121 | Name | : Suresh Kumar |
| 2122 | Designation | : Senior Scientist (Biotechnology) |
| 2123 | Division/Section | : Crop Improvement |
| 2124 | Location | : Indian Grassland and Fodder Research Institute (IGFRI) |
| 2125 | Institute Address | : Indian Grassland and Fodder Research Institute (IGFRI)<br>Gwalior road, Jhansi 284003 (UP) |

## 221 Project Technical Profile:

2211 Organization of Work Elements (For each objective and participating Investigator giving man-months involved) :

| Objective | Principal investigator (Man months) | Co-Investigator I (Man months) | Co-Investigator II (Man months) |
|---|---|---|---|
| To characterize the microsatellite (SSR) markers from cereal genomes and stress responsive ESTs by insilico mining | 6 | 0 | 0 |
| To study the cross transferability of microsatellite markers in grasses | 10 | 7 | 7 |
| To develop a forage genomic resource database | 6 | 0 | 0 |
| Total | 22 | 7 | 7 |

## 2212 Methodology:

### A) EST assembly and annotation

The Expressed sequences tags (EST) sequences were retrieved in the FASTA format from the NCBI database for further processing and clustering. Downloaded ESTs were subjected to sequence cleaning which involved removal of polyA/polyT tail, clipping low quality and low complexity sequences(<100bp), repeat masking and screening against the UniVec database available from NCBI (ftp://ftp.ncbi.nih.gov/pub/UniVec/) for contaminant vector and adapter sequences removal using the program Cross_Match. The deposited ESTs are often redundant in nature leading to over representation of sequence information. In order to remove the redundancy, EST sequences were clustered and assembled using the contig assembly program CAP3. The assembly results were in different output files e.g. unigenes, contigs and singlets. Clustered, non redundant contigs and singlet data sets were mined using Microsatellite identification tools, MISA and SSRIT using PERL scripts for the identification and localization of perfect and compound microsatellites. Microsatellites >10 bp with motifs ranging in length from 1 to 6 were considered. A minimum unit size of ten for mono-

**2213 Plan of Action:**

**Objective 1:** To characterize the microsatellite (SSR) markers from cereal genomes and stress responsive ESTs by insilico mining

a) Collection of stress responsive genes and ESTs available from literature/public databases (NCBI)

b) SSRs mining in Cereals (Rice/ Maize/Sorghum) genomes, stress responsive genes and ESTs.

c) In silico co-expression analysis to identify stress regulated genes

d) Primer designing for selected genes/ESTs and genomic regions

**Objective 2:** To study the cross transferability of microsatellite markers in grasses

a) Optimization of PCR conditions

b) Analysis of Polymorphic markers/alleles

c) Genetic Diversity analysis of germplasm

d) Electronic PCR for identification of orthologous SSR loci

f) DNA sequencing of polymorphic alleles

**Objective 3:** Development of a forage genomic resource database

a) Database design and development of integrated genome resource for nature and type of microsatellites, stress responsive networks, cross species transferable markers and phylogenetic profile of grasses

**2214 Time Schedule of Activities (Milestones):**

| Period of study | Milestone (Activity) |
|---|---|
| 6 Months | Retrieval of genomic information, stress responsive ESTs and their assembly into putative unigenes and contigs. Nature and type of SSR information in the data sets. Coexpression studies to identify set of candidate genes |

| | |
|---|---|
| 12 Months | Primer designing and synthesis, Electronic PCR to identify orthologues in other species and Optimization of PCR by touchdown profile. Design of Database |
| 18 Months | Cross transferability studies, identification and sequencing of polymorphic alleles and Genetic diversity analysis in *Sehima* sp |
| 24 Months | Cross transferability studies, identification and sequencing of polymorphic alleles and Genetic diversity analysis in *Heteropogon* sp |
| 30 Months | Cross transferability studies, identification and sequencing of polymorphic alleles and Genetic diversity analysis in *Dicanthium* sp |
| 36 Months | Development of a forage genomic resource database and report writing |

**2215 Annual Targets for Each Activity:**

| Period of study | Target |
|---|---|
| I$^{st}$ Year | Retrieval of genomic information, stress responsive ESTs and their assembly into putative unigenes and contigs. Nature and type of SSR information in the data sets. Coexpression studies to identify set of candidate genes<br>Primer designing and synthesis, Electronic PCR to identify orthologues in other species and Optimization of PCR by touchdown profile. Design of Database |
| II$^{nd}$ Year | Cross transferability studies, identification and sequencing of polymorphic alleles and Genetic diversity analysis in *Sehima* sp<br>Cross transferability studies, identification and sequencing of polymorphic alleles and Genetic diversity analysis in *Heteropogon* sp |
| III$^{rd}$ Year | Cross transferability studies, identification and sequencing of polymorphic alleles and Genetic diversity analysis in *Dicanthium* sp<br>Development of a forage genomic resource database and report writing |

## Part - V: DECLARATION

**This is to certify that:**

- the research work proposed in the Scheme/Project does not in any way duplicate the work already done or being carried out in the Institute on the subject ;
- the same Project has been/has not been submitted to any other Agency(ies) for financial support (if already submitted, identify Project & Agency) ; and
- the Investigator/Co-investigators have been fully consulted in the development of the Project and have fully undertaken their responsibility to carry out the programme as per the technical programme.

**Signature of the Project Investigator**:

**A.Radhakrishna**

**Co-investigators:**

9/11/12

**KK Dwivedi**

Suresh Kumar

**Suresh Kumar**

**Signature & Comments of the Head of the Division/Section :**

forwarded

12/11/12

**Signature & Comments of the Joint Director (Research):**

We may approve the Project

25/3/13

I/c PME

**Signature & Comments of the Director**:

25/3/13